Condition-dependence, colouration and growth of red eye combs in black grouse *Lyrurus tetrix*

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Abstract

Elaborate, condition-dependent ornaments function as sexual signals in many species, allowing individuals to gain higher dominance status or reproductive success. Ornament expression during peak-expression, i.e. the breeding season, is well-documented. However, dominance is often gained through year-round interactions and so signalling may be important prior to this. Only top quality males have the resources available to cope with prolonged investment into ornament growth and maintenance, so males of differing quality may have different strategies of ornament growth-scheduling. I investigated this in the lekking black grouse *(Lyrurus tetrix*), a species with multiple sexual signals, including red, integumentary eye combs. Eye comb size is dynamic and grows towards the breeding season. I tested whether condition- and dominance-related variables altered growth scheduling in the lead-up to (winter flocking) and during the breeding season. Heavier adult males, i.e. those with more resources, grew eye combs larger and faster. Dominant adults, in this case those with higher lek attendance, had larger eye combs at all times. This suggests growth-scheduling is condition-dependent but not dominance-related. I then investigated the interdependence of eye comb size with condition further, alongside its covariance with other morphological traits. Adults with larger eye combs had higher humoral immunity at the beginning of winter-flocking, which was reversed during the breeding season, and a near-significant positive association with parasite burden. Comb size is linked with testosterone, suggesting these males were experiencing immunological trade-offs, increasing condition-dependence during breeding. In addition, adults with larger eye combs also had the longest lyres, implying that only top quality males can simultaneously express multiple traits fully. The two signals may therefore function as multiple messages of quality, further enforcing honesty. Honesty in yearling sexual signalling was maintained by a lower level of immunological trade-offs and covariance with tail length. Therefore, only top quality males can afford the trade-offs associated with expressing larger eye combs, and maintain expression of multiple elaborate ornaments.

Additionally to the size component of ornaments, we also investigated colouration. Colouration is often measured using summary metrics, however colour is a product of the full spectrum so this approach is limiting. Comb redness arises from carotenoid-pigmented, red dermal spikes overlaying a white skin-based background. Comb enlargement is likely to 'spread' these spikes, affecting colouration. I used the whole colour spectrum to unravel the variation in colour attributable to condition-based variables. Males with larger combs were less red, but with more background reflectance. Heavier adults with a higher humoral immunity also showed more background reflectance. Hence, comb size is important in colouration, with condition-dependence being manifested in background reflectance. However, the composite nature of carotenoid-based colouration means variation to both components is likely to be important.

Overall, taking a multi-component viewpoint when studying ornament expression may be key to understanding condition-dependence and scheduling of sexual signal-development. Multiple traits and within-trait components, e.g. colour and size, are interlinked over prolonged periods in reflecting quality, and a full range of temporal and spectral data should be used to unravel this.

Thesis Outline

This thesis will be set out in five chapters. The first outlines the introduction and rationale of the research. Chapters 2-4 describe and discuss the methods and results of the project's three studies in detail. These chapters are written in the style of manuscripts and formatted for the Journal of Avian Biology, to be edited and submitted at a later date. The final chapter summarises and discusses the findings of the three studies as a whole, before concluding the thesis.

Contributions

I confirm that all stages of this Masters by Research degree are my own work, with contributions by Matti Kervinen (MK), Christophe Lebigre (CL), Tom Pike (TP), Carl D. Soulsbury (CDS) and the Life History Research in Ecology and Evolution research group (LyRE).

All chapters:

Data collection: 2001-13; LyRE, 2015; MK, **SH**

Project supervision: CDS, TP

Chapter 1: Introduction

Literature research: **SH**

Text: **SH**

Editing: TP, CDS, **SH**

Chapter 2: Condition and dominance-related patterns of sexual ornament growth in a lekking species:

Study design: CDS, **SH**

Data analysis: **SH**

Data interpretation: CDS, **SH**

Manuscript: **SH**

Manuscript editing: MK, CL, TP, CDS, **SH**

Chapter 3: Temporal covariance and interdependence of red eye combs with condition and sexual signals in black grouse *Lyrurus tetrix*:

Study design: CDS, **SH**

Data analysis: **SH**

Data interpretation: CDS, **SH**

Manuscript: **SH**

Manuscript editing: TP, CDS, **SH**

Chapter 4: The effects of condition-dependence on sexual ornament colouration across the spectrum

Study design: CDS, TP, **SH**

Data analysis: CDS

Data interpretation: TP, CDS, **SH**

Manuscript: **SH**

Manuscript editing: TP, CDS, **SH**

Chapter 5: General discussion

Text: **SH**

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Publications and Conferences

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Harris, S. H., Kervinen, M., Lebigre, C. L., Pike, T., Soulsbury, C. 2015. The effects of condition-dependence on sexual ornament colouration across the spectrum. **Paper – to be submitted.**

Ethics

Birds were captured under the permission of the Central Finland Environmental Centre (permissions KSU-2003-L-25/254 and KSU-2002-L-4/254), the Animal Care Committee of the University of Jyväskylä (ESLH-2009-05181/Ym-23) and the Ethics Committee of the University of Lincoln.

Chapter 1: Introduction

"There are, however, other sexual differences quite disconnected with the primary organs with which we are more especially concerned – such as the greater size, strength, and pugnacity of the male…his gaudy colouring and various ornaments, his power of song, and other such characters."

Darwin (1871) on male secondary sexual characters.

1.1 Sexual ornament expression

Elaborate, brightly-coloured or enlarged ornaments are common throughout the animal kingdom. Such secondary sexual traits have evolved through sexual selection acting to enhance traits that are attractive to females (intersexual selection), or to provide an advantage in male-male competition (intrasexual selection) where there is a competitive struggle for mates (Darwin 1859, 1871, Andersson 1994). There is wide evidence that sexual selection favours the evolution of phenotypically variable, energetically costly traits that only individuals in heritable, good quality can afford to express (see Johnstone 1995b for a review, Johnstone, Rands and Evans 2009), making them an honest signal of quality to conspecifics.

The costliness of ornamentation means many sexual traits are not constantly expressed. Many have an annual cycle, being developed to peak elaboration for the breeding season, and then contracted or lost afterwards; for example; many bird species have a pre- and post-nuptial moult around the breeding season; and most deer shed their antlers post-breeding and re-grow them over the summer, in time for the next breeding season (e.g. antlers; Chapman and Chapman 1982, Goss 1983, plumage; Marini and Durães 2001, Peters, Astheimer and Cockburn 2001). However, these traits are often fairly static, once grown, and so display a past reflection of male quality, i.e. during the time in which they were developed (e.g. Bartoš and Losos 1997, Keyser and Hill 1999,

Naguib and Nemitz 2007, Serra et al. 2007, Walker et al. 2013). For example, stress levels during moult correlate negatively with later reproductive success and survival in common eider ducks (*Somateria mollissima borealis*) (Harms et al. 2015). In contrast, skin-based (integumentary) ornaments are generally more continuously maintained, although not always fully expressed. Integumentary traits are also more dynamic, varying in colour or size in as little as seconds, according to changes in context (e.g. birds; Mougeot et al. 2010, Karubian et al. 2011, amphibians; Doucet and Mennill 2009, lizards; Velando, Beamonte-Barrientos and Torres 2006, fish; Rhodes and Schlupp 2012, mammals; Prum and Torres 2004). Dynamic, integumentary signals may therefore be a more reliable signal of current quality (Velando, Beamonte-Barrientos and Torres 2006, Pérez-Rodríguez 2008), which conspecifics may continually re-assess (Torres and Velando 2003).

Both static and dynamic traits have an important role to play in breeding season interactions, hence this is when ornaments are usually at their most elaborate (e.g. Negro et al. 1998, Griggio et al. 2007, Pérez-Rodríguez 2008, Horrocks, Perrins and Charmantier 2009). Evidence suggests that better quality males begin the breeding season with more elaborate ornaments, or develop them earlier, increasing reproductive success (e.g. Møller 1994, Green et al. 2000, Dobson et al. 2008). In more static ornaments, this may be facilitated by earlier or more accelerated growth (Barnard 1991, 1995, Bartoš and Losos 1997, Cockburn, Osmond and Double 2008, Ciuti and Apollonio 2011). Furthermore, many species also take part in intra or inter-sexual interactions outside of breeding, the outcomes of which may affect breeding-season success (e.g. Bearhop et al. 2004, Gunnarsson et al. 2005, Maia et al. 2012). More continuously expressed traits, i.e. integumentary characters, are likely to be important within such interactions, particularly in the pre-breeding season when competition intensifies, for example because territories are being assigned (McElligott, O'Neill and Hayden 1999, Pryke et al. 2002, Mougeot et al. 2005a, Schubert et al. 2007, Córdoba-Aguilar, Jiménez-Cortéz and Lanz-Mendoza 2009, Tonra et al. 2014). Despite this, studies of dynamic

ornaments are often carried out during peak expression (e.g. Mateos and Carranza 1995, Zuk et al. 1990, Jawor et al. 2004, Nooker and Sandercock 2008). Some empirical studies have investigated prolonged out-of-breeding dynamics of sexual signals, (e.g. Val, Queseda and Senar 2010, Vergara et al. 2012a and b, Martínez-Padilla et al. 2014), providing snapshots of variability in year-round expression, e.g. spring/winter or pre-/during/post-mating comparisons (McGraw et al. 2004, Mougeot, Redpath and Piertney 2005). In addition, previous theoretical modelling has unravelled some of the patterns associated with dynamic ornament development (e.g. Aparicio 2001, Lindström, Metcalfe and Royle 2005, Kokko 1997, Lindström et al. 2009), however there have been few empirical studies of continuous, pre-breeding growth trajectories of integumentary ornaments. The expression of an individuals' ornament and the information it conveys can change considerably over extended growth, due to changes in body condition, food availability and social status (Kitaysky, Wingfield and Piatt 1999, Cotton, Fowler and Pomiankowski 2004a, Jenni-Eiermann et al. 2007, Rubenstein and Hauber 2008, Karubian et al. 2011). The ability to cope with these changes and the costliness of extended signalling is expected to vary with male quality, affecting ultimate reproductive success (Morales et al. 2003, Dmitriew 2011, Rosenthal et al. 2012). Theoretical modelling suggests there should be clear differences between high and low quality males in growth-scheduling during the lead-up to the breeding season (Rands, Evans and Johnstone 2011), and I hope to empirically evidence this.

Costliness and condition-dependence of ornament expression

Ornament growth is assumed to be costly (Zahavi 1975, 1977), although the source of these costs is not always well characterised for a given trait. Despite this, several mechanisms have been shown to be common across species. Testosterone, for example, is often responsible for elaborate ornament growth, however, its immunosuppressive properties mean such displays may be traded-off with immunocompetence, and it is predicted only high quality males can cope with this (known as 'the immunocompetence handicap hypothesis' (ICHH); Folstad and Karter 1992, e.g. Peters 2000, Mougeot et al.

2004, but see Roberts, Buchanan and Evans 2004 for a review). In addition, the environment itself imposes costs, such as in the case of carotenoid-based colouration, which ultimately depends on carotenoid availability and foraging ability (Hill 1993, Hill and Montgomerie 1994, Smith et al. 2006, Pike et al. 2009). Carotenoids may also have an antioxidant or immunostimulant role, meaning that only high quality males can divert this resource towards the expression of brightly-coloured ornaments (known as 'the oxidation handicap hypothesis' (OHH); (Olson and Owens 1998, von Schantz et al. 1999, Martínez-Padilla et al. 2007, Sepp et al. 2011, Simons, Cohen and Verhulst 2012). However, these mechanisms need not be mutually exclusive; testosterone may in fact function as an oxidative stressor itself (von Schantz et al. 1999, Alonso-Alvarez et al. 2007, Mougeot et al. 2009), and may not always maintain honesty (see Johnstone and Grafen 1993, Searcy and Nowicki 2005, Velando, Beamonte-Barrientos and Torres 2014).

Overall, the immunological, physiological and energetic costs of ornament display mean that only those males in top phenotypic health can afford to trade-off resources for their expression, i.e. those in top condition (defined hereon as the pool of resources available to invest in allocation to life-history traits; Rowe and Houle 1996, Hunt, Brooks and Jennions 2005), rendering the ornament's expression condition-dependent, and making it an honest signal of quality (Zahavi 1975, Cotton, Fowler and Pomiankowski 2004a, Pérez-Rogríguez and Viñuela 2008). Those in top condition may have a set of specific, beneficial traits, such as disease-resistance or anti-predatory adaptations, which are likely to shift according to conditions (Hamilton and Zuk 1982, McCollum and Leimberger 1997, David et al. 2000, Garant, Sheldon and Gustafsson 2004, Andersson, Johansson and Söderlund 2006). Regardless, these characteristics should have a genetic basis, so that top traits are inherited by offspring, improving parental fitness, giving rise to competition in mate choice (Andersson 1994, David et al. 2000, Johnsen et al. 2003).

Expression of some sexual traits has, however, been shown to reflect quality by covariance with condition, as opposed to condition-dependence. These traits, for example melanin-based colours, have been shown to correlate with condition-based traits, such as

immunity, without relying on body condition (see Roulin et al. 2015 for a review), possibly as a result of pleiotropy (Fitzpatrick 2004). For example, in the polymorphic Eleonora's falcon (*Falco eleonorae*) darker nestlings have lower immunity than lighter morphs (Gangoso et al. 2011), hence colour and body condition appear to be associated. However, recent evidence suggests colour-based variation in this falcon is primarily attributable to a gene which also functions in immune responses, hence the two covary, and the trait is condition-related, not condition-dependent (Gangoso et al. 2015). Care must therefore be taken to avoid mistaken conclusions of direct condition-dependent associations from correlative studies (Roulin et al. 2015).

Many ornaments that are exaggerated in size are also brightly coloured. Therefore colouration may be important in the signalling function of dynamic, integumentary traits, e.g. larger collars in widowbirds (*Euplectes ardens*) are also redder (Andersson et al. 2002, also see Mougeot, Redpath and Leckie 2005, Mougeot 2008). Colouration has, in the past, often been studied using summary metrics, i.e. tristiumulus colour variables (Evans and Sheldon 2015, e.g. Serra et al. 2007, Murphy et al. 2009). However, final colouration is predicted to be a product of variation across the whole colour spectrum, making this approach limiting (e.g. Shawkey and Hill 2005, Caffrey et al. 2005, Griggio et al. 2009). Therefore, recently, novel methods have been developed that use data across the whole colour spectrum to model variation according to chosen factors, e.g. environment (e.g. Evans, Summer and Sheldon 2012, Evans and Sheldon 2013, 2015). Such methods have allowed a more objective, full picture of ornament reflectance and the effect of environmental or genetic variables to be visualised. However, they have not yet included directional impacts of variables in analysis, so it is not yet known how such variables may combine to affect final colouration.

Overall, there is a lack of study into the dynamics of individual ornament expression prior to the breeding season, specifically ornament growth-scheduling (Rands, Evans and Johnstone 2011). If prebreeding displays give a competitive advantage to species interacting during this time, for example by showing the ability to invest in

ornament display over extended time periods, then only top-quality individuals should be able to cope with costly displays on a prolonged basis. Therefore, male condition is likely to influence extended ornament expression, with the differences between males potentially giving rise to different strategies for scheduling ornament growth (Laucht, Kempenaers and Dale 2010, Rands, Evans and Johnstone 2011). In addition, many enlarged ornaments are also colourful, and so the ability to express brightly-coloured traits is likely to be important in affecting the ability of displays to improve success in intra- or inter-sexual interactions. Despite this, we have seen that methods used to measure colouration in relation to condition are often limited in their ability to understand the full range and effects of condition on final colouration, especially as colouration is often made up of variation across the spectrum, e.g. in carotenoid-based ornaments.

1.3 Research aims

In this thesis, I set out to investigate the growth patterns, covariance with condition and interdependence between sexual ornaments outside and during peak expression, using the red eye combs of black grouse (*Lyrurus* (formerly *Tetrao*) *tetrix*) as a model. I focus particularly on the winter flocking period leading-up to the breeding season, as this is when competitive interactions become to intensify. I begin with modelling the variation in scheduling of eye comb growth in relation to measures of condition and dominance status of males. I then focus on further unravelling the condition- and dominance-related trends found in the first investigation, and how these relate to other morphological traits outside of and during the breeding season.

In the final chapter, I look at the second major feature of red eye combs; carotenoid-based colouration, and its relationship with condition and eye comb size. I use and further develop a novel method to include analysis of the effect and direction of variables on final colouration, not just the proportion of variance attributable to them, and apply it using measures of condition, using the red, carotenoid-based eye combs as a model trait.

1.4 Study species

Black grouse were selected as a study species as they are a lekking bird, meaning that intra- and inter-sexual competition, and thus pressure from sexual selection on evolution of ornaments, is likely to be intense (Emlen and Oring 1977, Payne 1984). As a result, copulations are skewed to a small minority of top males (Alatalo, Höglund and Sutherland 1992, Kokko and Lindström 1997). Black grouse live in mixed-sex flocks in winter, which break down and become progressively more single-sex until the mating season in late April/early May (Kokko et al. 1998, Rintamäki et al. 1999, Siitari et al. 2007). For the purposes of ease within this thesis, the 'breeding period' was defined as during late April to early May, and the prebreeding period from January to early April. Black grouse are found in European taiga forests, adjacent to large open spaces used as lek sites (Lebigre et al. 2012). Few yearling males participate in dominance-related interactions, instead prioritising investment into development (Kervinen et al. 2015). Adult and yearling males were thus modelled separately for all analysis. The black grouse possess a suite of elaborate morphological and behavioural traits used in intense intrasexual and intersexual interactions, including an elaborate tail (lyre), blue chroma and red eye combs (Rintamäki et al. 2000, 2001, Siitari et al. 2007, Hämäläinen et al. 2012). The eye comb (Fig. 1.1) is

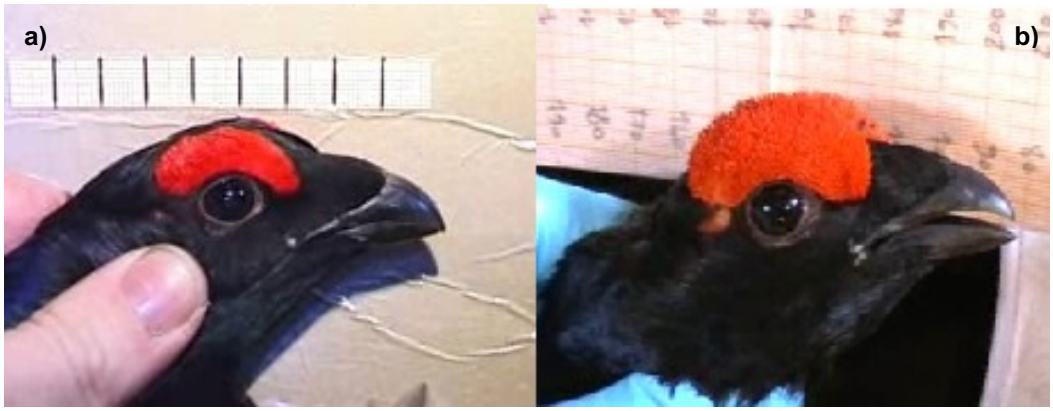

Figure 1.1: An individual male black grouse caught during the prebreeding (a – 30th January) and again during the breeding (b – 16th May) periods.

the most variable in size of these traits, changing in as a little as a few seconds, making it likely to act as a dynamic cue of quality (Rintamäki et al. 1999, 2000, Kervinen et al. 2015). Eye comb expression peaks during the breeding season, however, multi-annual, year-round interactions are important for dominance and copulatory success in black grouse, and so eye comb size may be expected to be important outside of this (Fig. 1.1) (Kokko et al. 1998, Rintamäki et al. 1999, Siitari et al. 2007). Its size is mediated by testosterone thus only high quality males can afford to grow large eye combs, making it a reliable cue of quality in intrasexual and intersexual interactions (Rintamäki et al. 2000). In addition, the colouration component of eye combs may also have a function in signalling quality, as its redness comes from carotenoid pigmentation (Mougeot, Redpath and Leckie 2005, 2007, Mougeot et al. 2007a, b, Pike et al. 2009).

Chapter 2: Condition and dominance-related patterns of sexual ornament growth in a lekking species

2.1 Introduction

Males of many species display elaborate ornaments which, if ornament production or maintenance comes at a cost to the bearer, function as honest indicators of individual quality (Zahavi 1975, Andersson 1994). These ornaments may function as intersexual signals; whereby females can assess and choose potential mates (Hill 1991), or intrasexual signals; whereby males can assess dominance in conspecific males (Chaine and Lyon 2008), or both (Griggio et al. 2007).

Many sexual ornaments go through cycles of renewal in an individual's lifetime; typically deer annually shed and regrow antlers, and birds moult and replace brightly coloured plumage. This means many ornaments are not displayed continuously, but grown expressly for the breeding season, and then moulted or contracted when no longer required (Jenni and Winkler 2011). Hence these signals reflect a temporally disjointed condition; for example plumage-based displays reflect quality during moult (e.g. McGraw et al. 2002, Serra et al. 2007, Harms et al. 2015). In contrast integumentary (skin-based) ornaments, such as wattles and combs, are expressed continuously and more dynamically, across the animal kingdom (e.g. fish: Pike et al. 2007; birds: Prum and Torres 2003, Rosenthal et al. 2012; reptiles: Langkilde and Boronow 2010). These structures can fluctuate to reflect changes in condition very rapidly; for example skin colouration in the blue-footed booby (*Sula nebouxii*) can vary according to nutritional condition as little as 48 hours (Velando, Beamonte-Barrientos and Torres 2006, also see Doucet and Mennill 2009, Butler and McGraw 2011). This means integumentary traits may act as a better cue of current phenotypic quality than ornaments grown on a longer term basis (Pérez-Rodríguez 2008, Hill, Hood and Huggins 2009).

Integumentary ornament size is typically mediated by androgens, which can act as immunosuppressants or oxidative stressors, making the signal physiologically or energetically costly to express (Folstad and Karter 1992, von Schantz et al. 1999, Alonso-Alvarez et al. 2007, Mougeot et al. 2009 and references therein). These ornaments, like other sexual signals, are usually largest when dominance is important, i.e. during the breeding season (e.g. Chapman and Chapman 1982, Goss 1983, Horrocks, Perrins and Charmantier 2009). However, many species interact outside of this, often particularly intensively in the months or weeks leading up to the breeding season, and these exchanges can affect ultimate dominance and reproductive success (e.g. Marra and Holmes 2001, Yoshino and Goshima 2002, Pryke et al. 2002, Mougeot et al. 2005a, Poisbleau et al. 2006, Reudink et al. 2009b). Most investigations have focussed on peak expression of dynamic ornaments (e.g. Faivre et al. 2001, Jawor et al. 2004, Murphy et al. 2009), with some assessing snapshots of inter-seasonal or longitudinal variation (e.g. Forstmeier 2002, Miller and Brooks 2005, Mougeot, Redpath and Piertney 2005), or correlates of dynamic ornament expresson with timing of breeding (Dobson et al. 2008), but few assessing continual integumentary trait development. We know that developing larger static (i.e. plumage or bone-based) traits earlier is costly but increases reproductive success, and may be facilitated by earlier and more accelerated growth (e.g. Dunn and Cockburn 1995, Barnard 1995, Bartoš and Losos 1997, Ninni et al. 2004, Siefferman, Hill and Dobson 2005, Serra et al. 2007). However, there is a lack of understanding of the growth trajectories and prolonged maintenance of more dynamic ornaments (e.g. Kokko 1997, Aparicio 2001, Lindström et al. 2009, Lee, Monaghan and Metcalfe 2012), especially during the pre-breeding period, and how this may give a competitive advantage (e.g. Barnard 1995, Peters, Astheimer and Cockburn 2001, Ciuti and Apollonio 2011, Rands, Evans and Johnstone 2011). Expression of these signals and the information conveyed by them during pre-breeding interactions can change dramatically, for example due to shifts in social status (Kitaysky, Wingfield and Piatt 1999, Setchell and Dixson 2001, Oliveira 2004), and conspecifics may dynamically assess this (Torres and Velando

2003). This growth period itself may even determine both the ultimate size of the ornament, and also its costliness, i.e. its condition-dependence (Rands, Evans and Johnstone 2011, Bartoš and Losos 1997). Previous theoretical modelling suggests these costs, combined with individual variation in phenotypic quality, may give rise to variability in scheduling of ornament growth (Rands, Evans and Johnstone 2011).

We investigated variation in ornament growth-schedules in black grouse (*Lyrurus tetrix*). The black grouse is a lekking, Galliform species with strong intersexual and intrasexual selection, in which copulations are skewed towards a few superior males (Alatalo, Höglund and Sutherland 1992, Kokko and Lindström 1997). Males have a number of morphological and behavioural traits that are used for both intersexual and intrasexual signalling (Hovi et al. 1994, Rintamäki et al. 1997, 2000, 2001, Siitari et al. 2007, Hämäläinen et al. 2012, Höglund, Johansson and Pelabon 1997, Lebigre et al. 2013) Of these, their testosterone-dependent red eye combs are the most variable in size (Rintamäki et al. 2000, Kervinen et al. 2015), peaking during the breeding season. Dominance is, however, acquired and maintained through multi-annual, year-round interactions (Kokko et al. 1998, Rintamäki et al. 1999, Siitari et al. 2007), suggesting that top males should have larger eye combs at all stages of growth. The breeding season occurs over approximately ~2 weeks during spring, but winter flocking and dominance-related interactions begin up to 3 months before this, during which time stress levels increase (Alatalo, Höglund and Lundberg 1990, Lebigre et al. 2012). Hence, associated energetic or physiological costs of ornament expression may mean that males varying in phenotypic quality differ in eye comb growth patterns. Using a longitudinal dataset of individual eye comb size across multiple years, we tested whether prebreeding eye comb growth patterns are related to condition and future male dominance.

2.2 Methods

Black grouse were studied at 5 lek sites in central Finland from January-May, 2001-13 and 2015. All sites are protected from hunting, suggesting the age and social structure of

the populations should be natural (lat. 62°15′N, long. 25°00′E). Individuals were caught (during January-April) with oat-baited walk-in traps, using standard protocols (see Siitari et al. 2007, Lebigre et al. 2012). Each individual was ringed, weighed (to nearest 10g) and aged as adult or yearling, based on plumage differences (Helminen 1963). Eye combs were recorded on a digital video camera against a scale, and then a still image was taken from the footage later on. The area of each eye comb (cm^2) was calculated using ImageJ software (Rasband 2012), and then summed to get total eye comb area for each individual (cm^2). During the mating season (~2 weeks in late April - early May) the sites were observed and behaviours of ringed males recorded daily, at regular intervals from 03:00 to 08:00 a.m. Recorded behaviours included; territory positions (distance from lek centre), lek attendance (in proportion to the highest attending male), and mating success (see Rintamäki et al. 2000 and Lebigre et al. 2012 for full methods).

Statistical Analysis

Growth scheduling of eye comb size (cm^2) was analysed using linear-mixed effect models (LMMs). In each model both linear and quadratic Julian date functions were included, to account for linear and non-linear eye comb growth. Within this model, we then tested four measures of male dominance: distance from lek centre (m), as dominant males hold territories closer to the lek centre; lek attendance, as dominant males attend the lek most often; copulations, as only the dominant males reproduce; and body mass (g), as heavier males have more resources to invest in ornament growth and dominance-related activities. Lek attendance was included as a binary factor, based on high (≥80%) and low (<80%) attendance, because of a sharp increase in mating success for those with ≥80% attendance (Kervinen et al. In press). Copulations were also analysed as a binary factor (0=no copulations, 1=copulated). To test for differences in growth scheduling, each measure was first tested in interaction with Julian date, and then as a fixed effect. In all models, individual identity, year and site were included as random effects. Adullts and yearlings were tested separately, as only a minority of yearlings participate in dominance-related behaviours, prioritizing investment instead into physical development, meaning

eye comb size may be less condition-dependent than in adults (Kervinen et al. 2015). Exact age was not included as an effect in adult males as they are all considered to be interacting as part of the lek. Models with a Gaussian error structure were all run in R version 3.1.2 (R Development Core Team 2012; R packages lme4 ver. 1.1-1.7, Bates, Maechler and Bolker 2015, and lmerTest ver. 2.0-2.5, Kuznetsova, Brockhoff and Christensen 2015).

2.3 Results

Adult eye comb growth-scheduling

Eye comb size was significantly related to the linear function of Julian date in adult males (Table 2.1). High lek attenders had significantly larger eye combs throughout the growth period, but did not grow them earlier or faster (Table 2.1a; Fig. 2.1a). Males closer to the lek centre showed a similar but near-significant trend (Table A1). Copulations were not significantly related to eye comb size (Table A1). In contrast, the interaction between eye comb size and body mass was significantly related to Julian date, both linear and quadratic terms (Table 2.1a). Heavier males began eye comb growth earlier, and grew them faster and larger (Fig. 2.1b).

Yearling eye comb growth-scheduling

In yearlings, eye comb size was also significantly related to yearling body mass, but only the linear function. Hence, heavier yearling males grew larger eye combs throughout the growth period, but with no variation in scheduling (Table 2.1b; Fig. 2.2). There was no relationship between eye comb size and lek attendance, distance from the lek centre or copulatory success.

Table 2.1: Significant LMM model outputs for the relationship between Julian date, and condition and dominance-related morphological and behavioural traits, with eye comb size for adult and yearling males. The full set of models is shown in Table A1. *Standard Error.

Model	Variable	Estimate	±SE*	*t*	*P*
	a) Adults				
Body mass (interaction) (233 individuals/ 345 points)	Intercept	21.86	27.84	0.79	0.433
	Julian date (linear)	-3.34	1.07	-3.13	**0.002**
	Julian date (quadratic)	0.02	0.00	2.74	**0.007**
	Body mass	-2.49	3.90	-0.64	0.524
	Julian date (linear) x body mass	0.46	0.15	3.09	**0.002**
	Julian date (quadratic) x body mass	0.00	0.00	-2.64	**0.009**
Lek attendance (fixed effect) (147 individuals/ 257 data points)	Intercept	3.97	0.29	13.73	**<0.001**
	Julian date (linear)	-0.04	0.01	-4.39	**<0.001**
	Julian date (quadratic)	0.00	0.00	11.23	**<0.001**
	Lek attendance	0.43	0.15	2.78	**0.006**
Distance from the lek centre (fixed effect) (142 individuals/ 245 data points)	Intercept	4.48	0.30	14.99	**< 0.001**
	Julian date (linear)	-0.04	0.01	-4.19	**<0.001**
	Julian date (quadratic)	0.00	0.00	10.61	**< 0.001**
	Distance from lek centre	-0.01	0.00	-1.88	0.062
	b) Yearlings				
Body mass (fixed effect) (751 individuals/ 759 data points)	Intercept	-12.36	4.21	-2.94	**0.003**
	Julian date (linear)	-0.04	0.00	-10.51	**<0.001**
	Julian date (quadratic)	0.00	0.00	20.55	**<0.001**
	Body mass	2.08	0.60	3.49	**0.005**

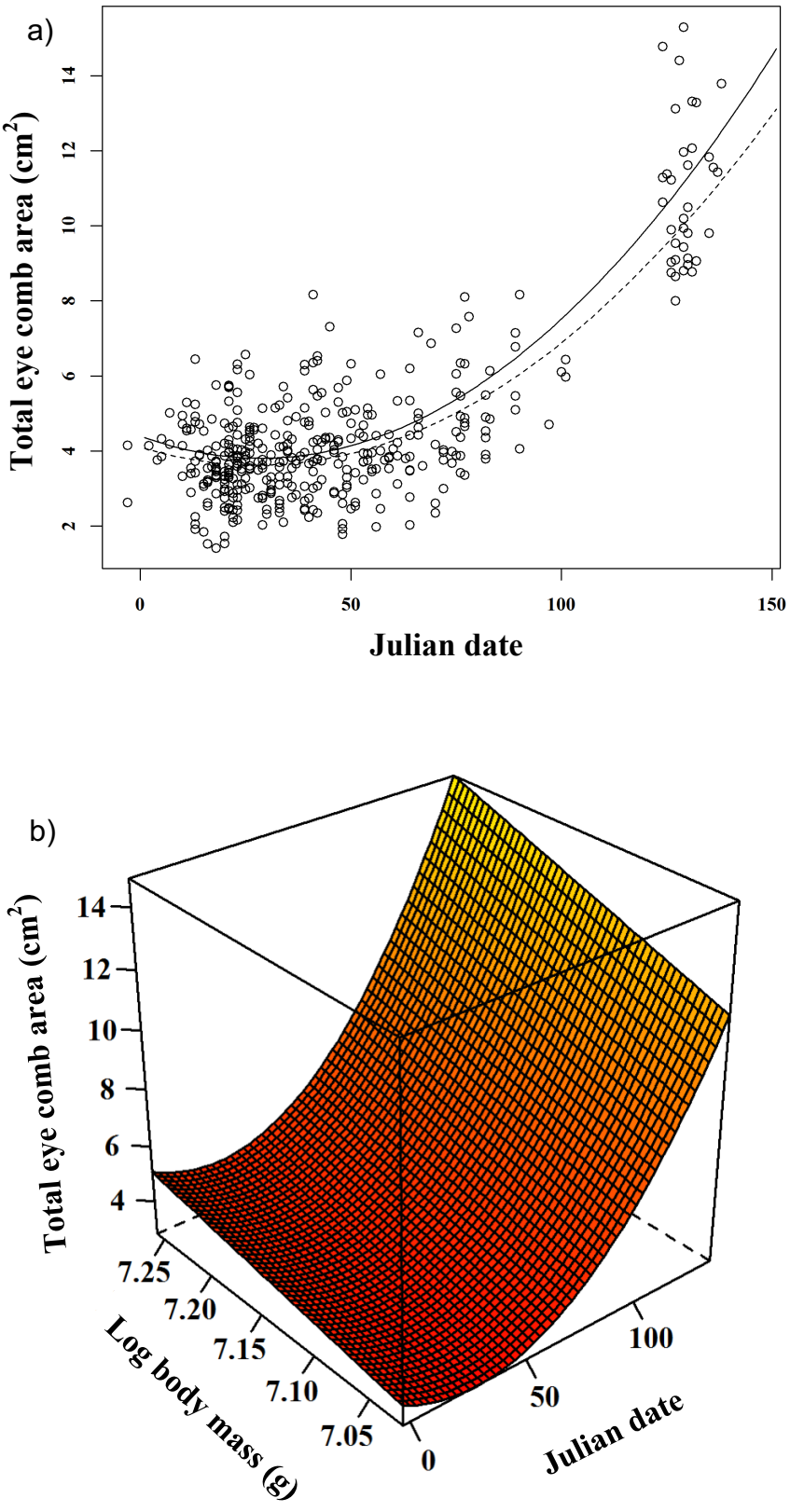

Figure 2.1: The relationship of adult eye comb growth (cm^2) with a) lek attendance and b) log body mass (g) over the Julian dateline of the prebreeding and breeding season. Lek attendance was measured as a binary variable; low attenders (≤80% attendance in proportion to highest attending male); dashed line, high attenders (≥80%); solid line.

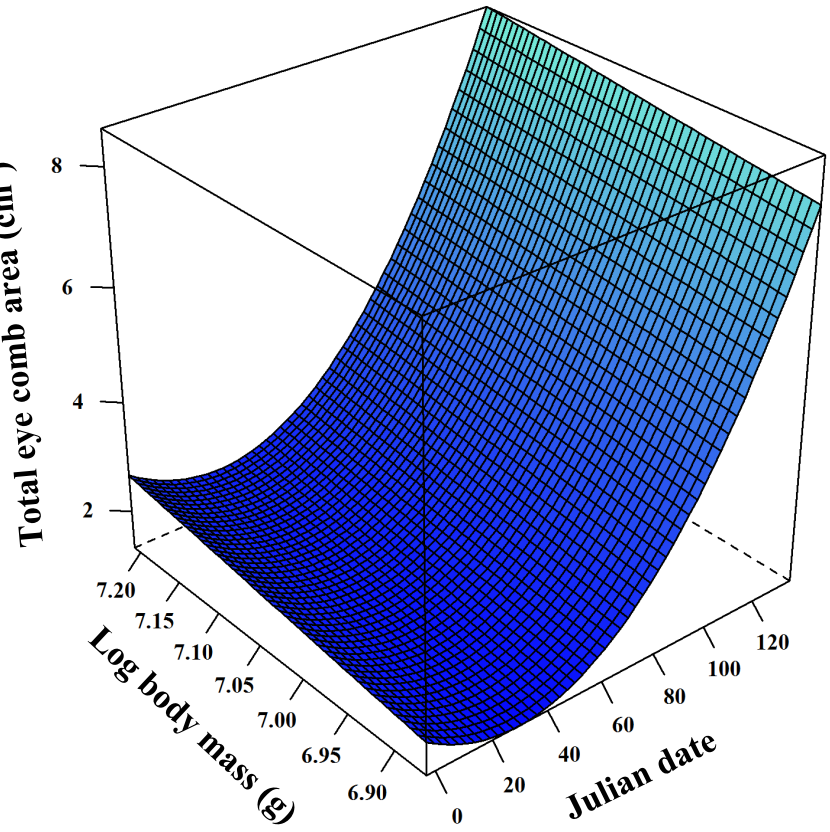

Figure 2.2: Eye comb growth (cm^2) in relation to yearling male Julian date with log body mass (g).

2.4 Discussion

Adult ornament growth-scheduling

Previous theoretical modelling of ornament-growth has suggested that earlier signal-bearing is negatively associated with quality, as a result of energetic costs (Rands, Evans and Johnstone 2011). Our results show that, at least in black grouse, the reverse is true. We found that there are major dominance and condition-dependent patterns of eye comb growth in the black grouse. Firstly, heavier adult males grew their eye combs earlier, faster and larger. Previous studies suggest that large ornament expression is condition-dependent (Holzer, Jacot and Brinkhof 2003, Cotton, Fowler and Pomiankowski 2004a, Poisbleau et al. 2006, Emlen et al. 2012, but see Badyaev and Duckworth 2003).

However, we extend this to suggest ornament growth patterns themselves are condition-dependent. High testosterone levels allow males to express larger eye combs and be more reproductively successful but also result in trade-offs between ornament expression and immune function or oxidative defence (Alatalo et al. 1996, Rintamäki et al. 2000, Mougeot et al. 2009, Martínez-Padilla et al. 2014). In addition, accelerated growth is likely to be more energetically costly, and may also be linked to oxidative damage, the effects of which higher quality males can better mediate (Mougeot 2009, see Dmitriew for a review 2011). Therefore, those individuals with more allocable resources available for investment into ornament growth, i.e. heavier black grouse males, are likely to better cope with the greater physiological investment that earlier or prolonged ornament growth entails (Møller 1994, Serra et al. 2007, Vannoni and McElligott 2009, Laucht, Kempenaers and Dale 2010, Yang et al. 2013).

We also found that more dominant males, with higher lek attendance, had larger eye combs throughout the growth period, but did not differ in growth-scheduling. In many species, dominance status is accrued or maintained through extended time periods, not just during the breeding season (Andersson 1989, Höglund, Johansson and Pelabon 1997, Friedl and Klump 2005, McElligott et al. 1998, McGraw et al. 2001, Poisbleau et al. 2006). Hence, ornament expression outside of the breeding season may have a function in social competition and, associatively, future reproductive success (Tibbetts and Safran 2009); for example in fallow deer (*Dama dama*), changes in male dominance status during antler growth affects antler growth rate (Bartoš and Losos 1997, Ciuti and Apollonio 2011). Investment into such periods of ornament development may further vary according to context, for example mate quality or intensity of intrasexual competition (e.g. Alatalo et al. 1992, Torres and Velando 2003, Wong and Svensson 2009). Regardless, having the resources to maintain a large ornament from earlier or growing it faster over an extended period, may have fitness benefits e.g. for successful establishment of territories or greater reproductive success (Barnard 1991, Møller 1994, Peters, Astheimer and Cockburn 2001, Pryke et al. 2002, Vannoni and McElligott 2009).

Taken together, our results suggest that having larger eye combs is important for dominance throughout growth, which is established through year-round interactions (Rintamäki et al., 1999, Kokko et al. 1999), but this does not impact growth-scheduling. Instead, it is the condition-dependence of ornament expression that is more importance for growth-scheduling, with males in better condition being able to cope with the costs of earlier ornament growth and prolonged maintenance (Barnard 1991, Laucht, Kempenaers and Dale 2010, Rosenthal et al. 2012).

Yearling eye comb growth-scheduling

Yearling males generally prioritise investment into development, and thus do not invest as highly into ornament expression as adults (Kervinen et al. 2015). Resultantly, yearling black grouse males have been shown to rank lower on the lek, achieving little reproductive success, with only a minority participating at all in dominance-related activities (Brittas and Willebrand 1991, Alatalo, Höglund and Sutherland 1992, Kokko and Lindström 1996). As such, in contrast to adults, we found that measures of dominance within yearlings were not significantly related to eye comb growth. Similar patterns are found in other species that invest in elaborate ornaments, for example in red deer (*Cervus elaphus*) allocation of investment to rutting and antler growth (both required for dominance) is lowest as a young male (Nussey et al. 2009, also see Balbontín et al. 2007, Evans, Gustafsson and Sheldon 2011). The evidence of low yearling investment is unsurprising when considering the significant carry-over costs that may result from investing highly into early, prolonged ornament growth, reducing later success in future ornament expression, reproduction and survival (Stearns 1989, Hunt et al. 2004, Siitari et al. 2007, Kervinen et al. 2015, see Metcalfe and Mongahan for a review 2003b). Hence, delayed ornament expression until full maturity, may, overall, be more beneficial to yearling lifetime reproductive success (Kervinen et al. 2015).

However, for those few males that do invest in lek displays, the condition-dependence of ornament expression tends to remain (Kruijit and Hogan 1967, Kervinen et al. 2012). Our results, similarly to previous results (Kervinen et al. 2015), showed that

heavier yearlings, like adult males, have larger eye combs but, unlike adults, growth-scheduling did not vary according to mass. Prolonged or faster ornament growth may be too costly for yearlings, however investing early into reproduction may be beneficial for future reproductive success, as females (both black grouse and other species) assess males based on their lifetime reproductive success (Kokko et al. 1999, Schubert et al. 2007), outweighing the negative effects of potential carry-over costs. Regardless, higher quality or better condition indvidiuals are more likely to have the resources available to invest in early or prolonged ornament expression (Lindström et al. 2009), a relationship which has been found across yearlings of several species (e.g. house finches; Badyaev and Duckworth 2003, bearded reedling (*Panurus biarmicus*); Surmacki et al. 2015). Hence, overall, condition-dependent ornament expression is continuous across both age groups.

2.5 Conclusions

We found that dominant adult males have larger eye combs throughout growth, but scheduling of growth may be condition-dependent. Being in top condition is likely to allow males to perform highly in year-round interactions, coping with prolonged expression. Extended growth and maintenance should allow males to improve or maintain dominance status, in order to increase future reproductive success. Further research into the variability of growth-trajectories according to context, e.g. mate quality or population density, may give further insights in to the condition-dependent scheduling of ornament growth. Overall, this suggests prebreeding growth may play a key part in future individual male dominance, and reproductive success.

Chapter 3: Temporal covariance and interdependence of red eye combs with condition and sexual signals in male black grouse *Lyrurus tetrix*

3.1 Introduction

The expression of costly, fitness-enhancing traits is usually limited by the allocable resources available for their production or maintenance, i.e. an individual's condition (Rowe and Houle 1996, Knell and Simmons 2010). Elaboration of the size or colour of such signals is often energetically costly; they may simply require resources to grow, be detrimental to their movement, or require diversion of investment away from somatic maintenance, e.g. immunostimulatory carotenoids being diverted to brightly-coloured signals signals (e.g. Pryke and Andersson 2005, Baeta et al. 2008). Consequentially, only high quality males are predicted to cope with the viability costs associated with displaying elaborate signals, thus enforcing honesty in the expression of intrasexual and intersexual signals in reflecting quality during male-male competition or female mate choice (Zahavi 1975, Andersson 1994).

However, there may be variation in the temporal period of condition indicated by an ornament; for example plumage-based traits are a reflection of past quality as they signal condition during the previous moult, and thus are temporally separated from the breeding season (Hill and Montgomerie 1994, Serra et al. 2007, Hegyi et al. 2007), even indicating average lifetime quality (Nowicki et al. 2000, Badyaev 2004). Other traits develop more quickly and dynamically, reflecting a more recent state of condition; individual ornament characteristics of deer (*Dama dama*) antlers, grown over the spring and summer, reflect dominance ranking during the time they were grown, as opposed to over the whole period (Bartoš and Losos 1997).

The most dynamic traits are integumentary, i.e. skin-based, traits which can respond to changes in context in as little as a few seconds (Rintamäki et al. 2000, Faivre

et al. 2003, Velando, Beamonte-Barrientos and Torres 2006, Rosen and Tarvin 2006). The elaboration of all ornaments is generally increased over a prebreeding period, reaching peak size or colouration for the breeding season (Bartoš and Loso 1997, Mougeot et al. 2005a, Maia et al. 2012), however integumentary ornaments may be dynamic and variable within this. Ornament expression, and the information it conveys, can change considerably over extended time periods, for example according to changes in dominance or condition (Kitaysky, Wingfield and Piatt 1999, Jenni-Eiermann et al. 2007, Karubian et al). 2011. For species in which intra- and inter-sexual interactions also take place outside of the breeding season, this period of prolonged prebreeding expression and conspecific interactions may be important in defining ultimate dominance and reproductive success (Green et al. 2000, Gunnarsson et al. 2005, Horrocks, Perrins and Charmantier 2009). Despite this, most studies have focussed on peak expression (Faivre et al. 2001, Nooker and Sandercock 2008, Murphy et al. 2009), with few considering integumentary ornament growth trajectories outside of this (e.g. Lee, Monaghan and Metcalfe 2012). Extended ornament expression is likely to be costly; many indviduals maintain high levels of testosterone to allow elaborate ornament growth and maintenance, however this also imposes negative immunological trade-offs (Folstad and Karter 1992, von Schantz et al. 1999, Mougeot, Redpath and Piertney 2005, see Dmitriew 2011 for a review). Prolonged expression itself may therefore be a costly signal of quality, particularly in the lead-up to the breeding season when competition intensifies (Zuk et al. 1998, McElligott, O'Neill and Hayden 1999, Pryke et al. 2002, Cockburn, Osmond and Double 2008). Resultantly, depending on the resources a male has available for ornament-investment, individuals may vary in integumentary ornament-growth or expression schedules according to quality (Kokko 1997, Aparicio 2001, Lindström et al. 2009), thus ornament growth should be expected to vary interdependently with measures of condition over the pre-breeding season (Rands, Evans and Johnstone 2011, chapter 2).

Furthermore, many species express multiple sexual ornaments simultaneously. Different types of ornaments, e.g. plumage-based and integumentary, may vary in the

investment and time required for their growth (Rosen and Tarvin 2006, Vergara and Fargallo 2011). This means that the covariance between ornaments may also be expected to vary across the breeding season, with only the highest quality males being able to express multiple traits simultaneously. Few studies have considered the variation of dynamic ornament growth trajectories outside of the breeding season, in the context of condition and expression of other morphological traits (e.g. Rands, Evans and Johnstone 2011, Laucht, Kemenaers and Dale 2010, chapter 2). For species displaying multiple sexual traits, analysing these relationships may be key to understanding how the costliness and scheduling of ornament expression affects ultimate dominance and reproductive success (Johnstone, Rands and Evans 2009, Rands, Evans and Johnstone 2011), and we look to investigate this.

To study the trajectory of ornament expression with condition and multiple traits, we used eye combs in the black grouse *Lyrurus tetrix* as a model trait. We modelled growth of this integumentary sexual ornament from the prebreeding period, through to the breeding season using a longitudinal dataset containing morphological and condition-based measures. Black grouse are a lekking Galliform species with a suite of sexual-selected morphological and behavioural traits, which function in intrasexual and intersexual signalling (Rintamäki et al. 2000, 2001, Siitari et al. 2007, Hämäläinen et al. 2012). Their red eye combs are the most size-variable of all their traits, and are costly to express as a result of their testosterone-dependence (Rintamäki et al. 2000, Kervinen et al. 2015). We have shown previously that eye comb growth is body condition-dependent and dominance-related (chapter 2). Black grouse, like many species, maintain social dominance through year-round interactions (Rintamäki et al. 1999, Kokko et al. 1999, Schubert et al. 2007, Reudink et al. 2009a), suggesting traits should be condition-dependent at all times. However, as signals can be used for female choice (e.g. Kodric-Brown and Nicoletto 2001), their condition-dependence may be exaggerated during the breeding season. We therefore modelled the (a) dependence of eye comb growth on health-related condition measures; immunoglobulin concentration, parasite burden and

body mass, and (b) relationship of eye comb size to other condition-dependent traits; lyre (tail) length, wing length and body mass, during the prebreeding season in male grouse, with the expectation that condition-dependence would vary with time, condition and other sexual traits.

3.2 Methods

Black grouse are a lekking species which occupy Eurasian taiga forests, gathering in open areas with a forest edge to lek, flocking during the winter months, and then breeding in late April/early May (Siitari et al. 2007, Lebigre et al. 2012). Birds were studied at 5 peat bog lek sites located around Petäjävesi, central Finland (lat. 62°15′N, long. 25°00′E). All sites are protected from hunting, suggesting a natural population structure. Birds were captured using baited walk-in trips during the winter flocking seasons (January – May) in 2001-13 and 2015, using standard protocols (Alatalo et al. 1996, Siitari et al. 2007, Lebigre et al. 2012). Each individual was ringed with a coded metal ring, before taking a range of morphometric and health-based measurements.

Morphological measures

For each bird, age was estimated as adult (≥2 years old) or yearling (1 year old), based on plumage colour and morphology differences (Helminen 1963). Birds were then weighed (to nearest 10g) and measured for wing length (mm), maximum tail feather length (lyre) (mm), and tarsus (mm). A digital video recording was also made of both eye combs for each bird against a known scale. A still image of each comb was taken from this footage and imported into ImageJ software to calculate the area of each eye comb (cm^2) (Rasband 2012), before totalling them to give total eye comb size (cm^2).

Condition measures

Microfilaria burden and concentration of plasma immunoglobulins (IgGs) were the two measures chosen for condition analysis. IgGs form part of the humoral immune response and, on detection of foreign substances, production of antibody-specific IgGs is triggered

by the host (Lebigre, Alatalo and Siitari 2013). At each capture, replicates of 75 µl and 1-2 ml samples of blood were taken and analysed using the methods and timings outlined in Lebigre, Alatalo and Siitari (2013). To summarise, samples were first centrifuged (12,000 rpm, 5 minutes) to separate plasma and red blood cells. A proportion of plasma was frozen for later immunoglobulin analysis, and the rest of the samples were analysed immediately for parasites. Parasites were counted at the interface between plasma and erythrocytes for 2 replicates, under a microscope. The same observer analysed both samples, and analysis showed high repeatability between replicates. *Microfilaria* has been found to be one of the most abundant parasites in black grouse, and thus will be used as a measure of parasite burden in this study (Höglund, Alatalo and Lundberg 1992, Lebigre, Alatalo and Siitari 2013).

Plasma IgG concentration was measured using the ELISA method, full details of which are described by Pihlaja et al. (2006) and Lebigre, Alatalo and Siitari (2013), where full methodological details can be found. ELISA plates were coated with anti-chicken IgG antibody and refrigerated overnight. Plates were then emptied and saturated with 1% bovine serum albumin prepared in a neutral phosphate-buffered saline and washed with PBS-Tween. Samples, along with their replicates and standards were then prepared at dilutions of 1: 40,000 and 1:80,000 in 1% BSA-PBS and left to incubate for 3 hours. The plate-washing process was repeated and an alkaline phosphatase conjugated IgG was added, and samples once again refrigerated overnight. Plates were washed one final time before adding an alkaline phosphatase substrate in an amine buffer to each well, before leaving for a 45 minute incubation period. Spectrophotometry was then used to calculate the optical density of each well at 405 nm, using a curve generated by the standards for calibration.

Statistical analysis

Variation in the combined size of both eye combs (cm^2) was modelled using linear-mixed effect models (LMMs). We analysed adults and yearlings separately because age-specific growth of eye combs and other traits is highest between ages 1 and 2, and allocation of

resources to reproduction is much lower in yearlings (Kervinen et al. 2012, 2015), meaning that condition-dependent signalling may be less important at this age. We used an AIC-IT (Akaike's Information Criterion-Information Theoretic) approach (Burnham and Anderson 2002) to assess the relative importance of individual parameters in eye comb growth. Two basic global models were created, the first including condition measures (hereafter "condition model"); *Microfilaria* count (1+logged), logged IgGs (s.u.) and body mass (g) and the second including phenotypic measures (hereafter "phenotype model"); body mass (g), maximum lyre length (mm) and wing length (mm). Body mass was included in both models as it is a recognised reflection of both health status (Alonso-Alvarez and Tella 2001) and phenotypic quality (Iyengar and Eisner 1997, Welch, Semlitsch and Gerhardt 1998). In terms of the rationale for choosing parameters, firstly, the measures used in the condition model are well-documented in assessing health status. In the phenotype model; maximum lyre length is a sexual trait known to reflect male quality and is used in mate choice. Wing length is not generally considered a sexual trait, however it may be correlated with male quality, and comparisons between non-sexual and sexual traits can be useful to ensure the latter is more significantly related to condition or quality (Møller and Pomiankowski 1993, Palmer and Dingle, 1986, Cotton, Fowler and Pomiankowski 2004b). For all models each condition or phenotypic parameter was tested in interaction with both linear and quadratic Julian date, with total eye comb size as the dependent variable. The relationship between eye comb size and Julian date (both functions) is significant and thus Julian date, and its relationship with comb size, will be significant and highly important in all models (Table 2). In all LMMs, individual identity was also included for as a random effect, to account for non-independence of data. LMMs with a Gaussian error structure were all run using packages lme4 ver. 1.1-1.7 (Bates, Maechler and Bolker 2015) and lmerTest ver. 2.0-2.5 (Kuznetsova, Brockhoff and Christensen 2015) in R version 3.1.2 (R Development Core Team 2012).

Models were first standardized to a mean of 0 and standard deviation (SD) of 0.5 (using arm R package ver. 1.8-6, Gelman et al. 2015), in order to relativize parameter

estimates for comparison after model averaging. Model selection was then carried out using the dredging function within the MuMIn R package ver. 1.15.1, calling for all possible model combinations based on the global models (Bartoń 2015). A confidence set of models was calculated using a model selection by ranking the top models from largest to smallest, and selecting the set that had a summed Akaike weight of 95%+ (Burnham and Anderson 2002, Grueber et al. 2011). Model averaging was carried out on these models based on the methods outlined in Grueber et al. (2011) in order to obtain parameter estimates, relative importance and confidence intervals for each condition measure. This approach allows model uncertainty to be taken into account, in order to carry out more robust modelling (see Whittingham et al. 2006). The final models, those with confidence intervals non-overlapping 0, were then considered to be the significant global models for eye comb growth.

3.3 Results

Adult males

Within the condition model, the most important significant variables were IgGs, body mass, the interaction between Julian date (linear) and *Microfilaria* (Table 3.1a). In addition, the positive interaction between *Microfilaria* and Julian date (quadratic) was approaching significance, but of lower relative importance (Table 3.1a). Males with larger eye combs had a higher IgG concentration at the beginning of the breeding season, however this changed in the breeding season, with those growing larger eye combs having fewer IgGs (Fig. 3.1a). Both models suggested that heavier males grew larger eye combs throughout the prebreeding and breeding season. This trend was stronger in the phenotype model, and thus the graph produced is based on this (Table 3.1 and b; Fig. 3.1b).

Body mass, maximum lyre length and the interaction between maximum lyre length and Julian date (quadratic) were the most important factors in the phenotype model (Table 3.1b). However, individuals with longer lyres grew their eye combs faster and

larger than those with smaller lyres (Fig. 3.1c). In the breeding season, there was much stronger covariance between lyre length and eye comb size than during the prebreeding season.

Table 3.1: Relationship of condition and quality-based parameters with total eye comb size and Julian date in adult males. (*Julian date. **Immunoglobulin concentration (s.u.) (IgG)). Results in emboldened text marks those considered as significant, as confidence intervals are not overlapping 0.

Parameter	Estimate	95%CI (lower, upper)	Relative importance
a) Global condition model (254 individuals/328 data points)			
Julian date (quadratic)	**2.75**	**(2.40, 3.10)**	**1.00**
Julian date (linear)	**0.74**	**(0.40, 1.08)**	**1.00**
IgG (s.u.)*	0.19	(-0.06, 0.43)	1.00
Body mass	**0.47**	**(0.23, 0.71)**	**1.00**
Julian date (linear) x IgG	**-0.81**	**(-1.24, -0.34)**	**1.00**
Microfilaria	0.06	(-0.21, 0.33)	0.53
Julian date (quadratic) x *Microfilaria*	0.46	(-0.05, 0.97)	0.28
Julian date (linear) x *Microfilaria*	0.46	(-0.11, 1.03)	0.26
Julian date (quadratic) x IgG	0.22	(-0.37, 0.80)	0.24
Julian date (quadratic) x Body mass	-0.21	(-0.72, 0.30)	0.20
Julian date (linear) x Body mass	0.07	(-0.57, 0.70)	0.18
Julian date (quadratic)	**1.89**	**(1.70, 2.09)**	**1.00**
b) Global phenotype model (525 individuals/764 data points)			
Julian date (linear)	**0.76**	**(0.53, 0.99)**	**1.00**
Maximum lyre length (max. lyre)	-0.14	(-0.34, 0.05)	1.00
Body mass	**0.52**	**(0.37, 0.72)**	**1.00**
Julian date (quadratic) x max. lyre	**0.61**	**(0.34, 0.89)**	**1.00**
Julian date (linear) x max. lyre	-0.16	(-0.63, 0.31)	0.20
Julian date (linear) x body mass	0.17	(-0.24, 0.58)	0.17
Wing length	0.09	(-0.11, 0.29)	0.12
Julian date (quadratic) x body mass	-0.11	(-0.48, 0.26)	0.12
Julian date (quadratic) x wing length	-0.29	(-0.64, 0.07)	0.04
Julian date (linear) x wing length	0.03	(-0.42, 0.48)	0.02

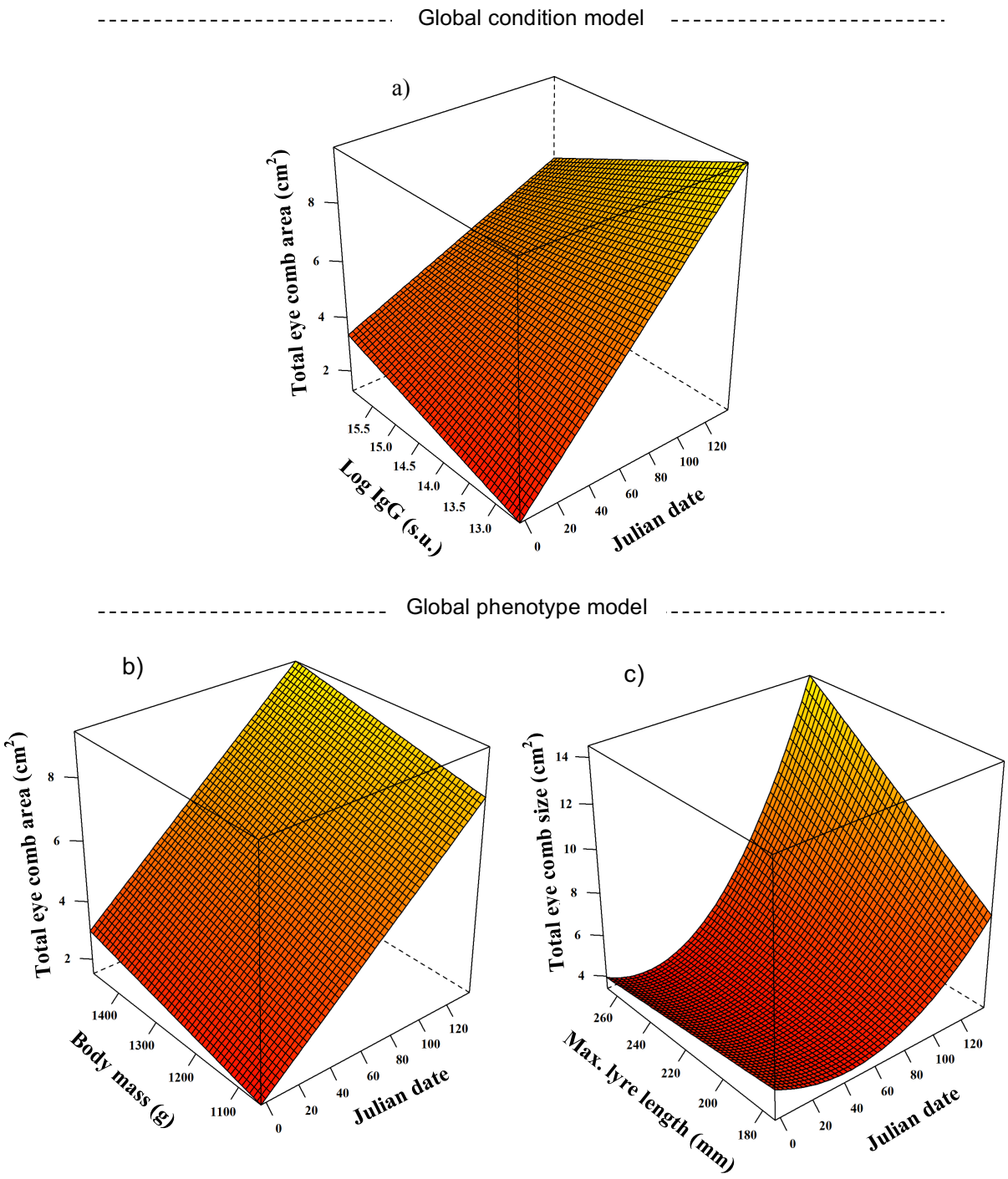

Figure 3.1: The interdependence and covariance of adult eye comb growth (cm^2) with condition, modelled by a) IgG concentration (s.u.) in a global condition model, and other morphological traits b) body mass (g) and c) maximum lyre length (mm), modelled in a global phenotype model, over the course of the pre-breeding and breeding seasons.

Yearling males

Body mass, *Microfilaria*, the interaction between *Microfilaria* and Julian date (linear), and the interaction between body mass and Julian date (linear and quadratic) were the most relatively important variables within the global condition model (Table 3.2a). Of these, all were significant and, in addition, IgGs with Julian date (quadratic) were also significant, but with a lower relative importance. In contrast to adults, yearlings growing eye combs larger and faster had higher IgG levels (Fig. 3.2a) and lower *Microfilaria* counts (Fig. 3.2b). Conversely to adults, lighter males grew larger eye combs over the prebreeding season in both models, however this trend was stronger in the phenotype model, and thus the graph shown is based on this modelset (Table 3.2; Fig.3.2c).

In the global phenotype model, the variables with the highest relative importance values were body mass, wing length, the interaction between body mass and Julian date (quadratic) and maximum lyre length (Table 3.2b). In those variables not significant at a temporal level, i.e. non-interactions, eye comb size and wing length had a negative association (Fig. 3.2d), whilst maximum lyre length and eye comb size were positively associated (Fig. 3.2e). The graphical representations of these latter two results (Fig 3.2) still include Julian date, due its positive relationship with eye comb size.

Table 3.3: Relationship of condition and quality-based parameters with total eye comb size and Julian date in yearling males. (*Julian date. **Immunoglobulin concentration (s.u.) (IgG)). Results in emboldened text marks those considered as significant, as confidence intervals are not overlapping 0.

Parameter	Estimate	95%CI (lower, upper)	Relative importance
a) Global condition model (391 individuals/ 396 data points)			
Julian date (quadratic)	**0.98**	**(0.83, 1.14)**	**1.00**
Julian date (linear)	**0.29**	**(0.11, 0.46)**	**1.00**
Body mass	**0.48**	**(0.30, 0.66)**	**1.00**
Microfilaria	**0.16**	**(0.01, 0.31)**	**0.96**
Julian date (linear) x *Microfilaria*	**-0.56**	**(-0.90, -0.23)**	**0.94**
Julian date (quadratic) x Body mass	**-0.68**	**(-1.16, -0.19)**	**0.88**
Julian date (linear) x Body mass	**0.37**	**(0.002, 0.74)**	**0.51**
IgG	-0.10	(-0.28, 0.08)	0.23
Julian date (quadratic) x IgG	**0.62**	**(0.15,1.09)**	**0.19**
Julian date (quadratic) x *Microfilaria*	0.12	(-0.25, 0.50)	0.15
Julian date (linear) x IgG	0.11	(-0.28, 0.50)	0.03
b) Global phenotype model (722 individuals/729 data points)			
Julian date (quadratic)	**1.18**	**(1.04, 1.32)**	**1.00**
Julian date (linear)	**0.49**	**(0.33, 0.64)**	**1.00**
Body mass	**0.32**	**(0.18, 0.47)**	**1.00**
Wing length	**-0.21**	**(-0.35, -0.07)**	**0.94**
Julian date (quadratic) x body mass	**-0.35**	**(-0.64, -0.07)**	**0.73**
Max. lyre	**0.15**	**(0.02, 0.27)**	**0.51**
Julian date (quadratic) x wing length	-0.02	(-0.47, 0.44)	0.15
Julian date (linear) x wing length	0.11	(-0.25, 0.47)	0.15
Julian date (linear) x body mass	0.02	(-0.31, 0.34)	0.11
Julian date (linear) x max. lyre	-0.14	(-0.39, 0.11)	0.08
Julian date (quadratic) x max. lyre	-0.01	(-0.25, 0.23)	0.03

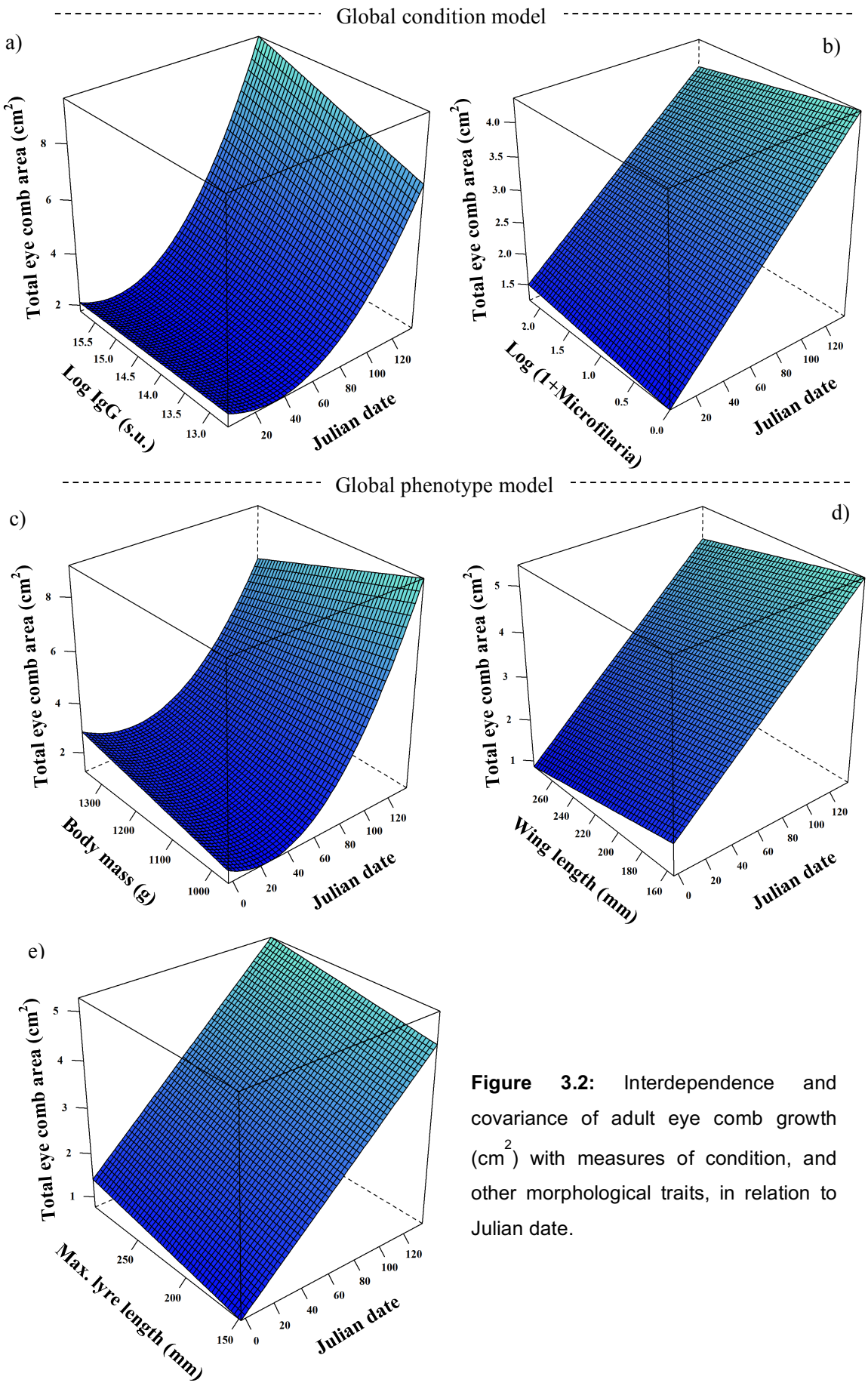

Figure 3.2: Interdependence and covariance of adult eye comb growth (cm^2) with measures of condition, and other morphological traits, in relation to Julian date.

3.4 Discussion

Adults and eye comb size

In many species, sexual ornaments reliably indicate some component of male quality. Elements of ornament elaboration, such as size or colouration, have been shown to reflect territory quality, nutritional status and parasite load (Salvador et al. 1996, Keyser and Hill 2000, Pérez-Rogríguez and Viñuela 2008). Unsurprisingly, in adult black grouse, eye combs showed a strong relationship with components of condition. Firstly, in line with previous work (chapter 2), heavier males grew large eye combs. Ornaments in general are costly to produce, so heavier males are advantaged by having more resources available to invest, a relationship seen throughout the animal kingdom (e.g. pied flycatchers *Ficedula hypoleuca*; Lampe and Espmark 1994, fallow deer; McElligott et al. 2001, field crickets *Teleogryllus commodus*; Hunt et al. 2004). In addition, ornament costliness increases with the pressures of prolonged expression, so the ability to cope with long-term investment may itself be a signal of quality (e.g. Kokko et al. 1999, Rintamäki et al. 1999, McGraw and Gregory 2004, Cockburn, Osmond and Double 2008, Vannoni and McElligott 2009, Rands, Evans and Johnstone 2011, chapter 2). This suggests only males in superior body condition can cope with prolonged displays.

Eye combs, like many ornaments (e.g. Salvador et al. 1996), are testosterone-dependent (Ligon et al. 1990, Rintamäki et al. 2000, Mougeot et al. 2004, Yang et al. 2013). Whilst having high levels of testosterone enables the growth of larger ornaments, its immunosuppressive qualities lead to trade-offs with immune function (Folstad and Karter 1992, von Schantz et al. 1999, Alonso-Alvarez et al. 2007, Mougeot et al. 2009). We found strong evidence for exactly this; males with large eye combs had higher IgG levels prior to the breeding season, but lower IgGs during the breeding season i.e. when testosterone levels were highest. This suggests males may lose condition during sexual interactions (Zuk et al. 1998, Vannoni and McElligott 2009), and the condition-dependence of eye comb expression may become heightened when physiological limits

are under pressure, i.e. during the breeding season when more intense sexual interactions (fighting or displaying) are taking place, and therefore higher ornament investment may be required (Cotton, Fowler and Pomiankowski 2004a, Vergara et al. 2012a and b).This may mean the signal becomes more honest over the course of the prebreeding and breeding season (Lindström et al. 2009). In turn, there was a positive trend of males with more elaborate eye combs having a higher parasite burden, likely as a result of reduced or suppressed humoral immunity (Barber et al. 2001, Kilpiamaa, Alatalo and Siitari 2004). This result was, however, non-significant and the *Microfilaria* parasite was generally of low importance in adults, although a relationship between the two measures has been previously suggested (Lebigre, Alatalo and Siitari 2013). The non-significance of this result may be because *Microfilaria* is simply not be a key parasite for black grouse; there are multiple other blood and gut parasites that may be important for this species (Lebigre, Alatalo and Siitari 2013; Soulsbury, unpublished data). These results further suggest, alongside previous study, that males of better condition can cope with extended ornament expression to gain a competitive advantage (chapter 2). The evidence of these immunocompetence trade-offs, likely mediated by testosterone, builds on previous results (chapter 2) in suggesting the mechanisms by which this signal is costly.

Adults and multiple traits

Many species have evolved multiple sexual signals that are used for female mate choice (Candolin 2003) or in male-male competition (Vanpé et al. 2007). Our results show covariance of eye comb size with another sexual trait, lyre length, suggesting the suite of sexual signals seen in black grouse may be acting as multiple cues of quality. Adult males with longer lyres grew their eye combs faster, with a stronger relationship between lyre length and eye comb size during the breeding season, when eye combs are at their largest. Similarly to eye combs, plumage-based traits, such as lyre length, are costly to grow and maintain (Pryke and Andersson 2005, Muñoz, Aparcicio and Bonal 2008). Those with longer tails are more reproductively successful and immunocompetent,

suggesting they are better able to survive and cope with the costs of elongated tail length (Saino, Møller and Bolzerna 1995, Saino and Møller 1996, Kleven et al. 2005). This suggests that the two differing traits, plumage-based and integumentary, act together as signals of phenotypic quality (e.g. Zuk et al. 1990, Hill, Hood and Huggins 2009). Heightened breeding-season covariance between the two ornaments further enforces honesty, with only top males having the resources to cope with expressing the most exaggerated of both ornaments. The non-significance of covariance between wing length and eye comb size was unsurprising, as it is a non-sexual trait and unlikely to be important in the breeding season, or highly variable with condition. This further supports lyre length as being a sexual trait, having a more heightened relationship with other signals, than that of the non-sexual trait (Cotton, Fowler and Pomiankowski 2004a).

The two traits are grown on very different time scales, and thus are likely to represent different messages about male quality (e.g. Kodric-Brown and Nicoletto 2001, Doucet and Montgomerie 2003, Vergara and Fargallo 2011). The two ornaments may function as backup signals, whereby multiple signals reflect the same quality with some error (Candolin 2003); for example, peacock (*Pavo cristatus*) plumage and behavioural displays both indicate health, but the former indicates current condition, whereas the latter indicates potential immunological response (Loyau et al. 2005, also see Hill, Hood and Huggins 2009). Alternatively the two traits may reflect condition at different time periods, i.e. past and present, where context, such as nutritional resources available, may have differed (Scheuber, Jacot and Brinkhof 2003, Siepielski, DiBattista and Carlson 2009, Maia et al. 2012). Growing ornaments faster is likely to be energetically costly, so the trend of better condition males growing eye combs faster may be a signal of current viability, with lyre length representing past investment in signalling (Barber et al. 2005). In this way the signals also combine to reflect long-term, as well as present, investment into signal expression (Saks, Ots and Hõrak 2003, Lebigre, Alatalo and Siitari 2013), and all of the signals may, therefore, be used in combination by conspecifics to assess quality (Mateos and Carranza 1995).

Alternatively, lyre length and comb size may give multiple messages, whereby each signal gives different information (Johnstone 1995a, McGraw and Hill 2000, Candolin 2003, Bro-Jørgensen and Dabelsteen 2008, Vergara and Fargallo 2011); in barn swallows (*Hirundo rustica*), tail length indicates survival prospects and immune response, whereas its song represents social status (see Saino et al. 2003). Each signal may even be giving information to different receivers ('multiple receiver hypothesis'; Andersson et al. 2002). The exact information reflected by lyre length and eye combs is unclear but, regardless, the heightened covariance between these traits during the breeding season suggests they act as multiple signals to reliably cue individual quality.

Yearling males and eye comb size

The majority of adult males invest in lekking activity, whereas significantly fewer yearlings do so (Kervinen et al. 2012). Yearling males face different resource-investment trade-offs in comparison to adults, as they are still undergoing considerable physiological and physical development between years 1 and 2 (Kervinen et al. 2015). Hence, yearling black grouse, like young males of many species, do not invest as greatly into sexual signalling as adults (Slagsvold and Lifjeld 1985, Kokko 1997, Préault et al. 2005, Lifjeld et al. 2011, Kervinen et al. 2012, 2015). In contrast to adults, yearlings with large eye combs during the breeding season had higher IgGs and a lower *Microfilaria* count than yearlings with smaller eye combs. This is likely because young males have lower testosterone levels (Alatalo et al. 1996, Siitari et al. 2007), meaning trade-offs with immunity are reduced. Young individuals are also generally less parasitized than adults, possibly because parasite intensity increases with age and exposure to infection (Skarstein and Folstad 1996, Mougeot, Evans and Redpath 2005). However, for those yearlings that had been infected, parasites were important; suggesting any infection in yearlings has a significant impact on condition, and ability to express ornaments.

Surprisingly, lighter males had larger eye combs during the breeding season. Body mass itself is a dynamic trait and, in displaying adult males, it generally declines over the breeding season (Mitchell, McCowan and Nicholson 1976, Höglund, Kålås and Fiske

1992, Mougeot, Redpath and Piertney 2006, Lebigre, Alatalo and Siitari 2013). The same may be true for yearlings, meaning that those who do invest in lekking and grow large eye combs may lose, or have lost, condition very rapidly, even being in lower condition to begin winter flocking with. Hence, these results suggest yearling eye comb size covaries with condition too.

This is further supported by the positive association of eye comb size with maximum lyre length in yearlings. It is likely that only higher quality yearling males would be able to afford the viability costs of longer tails at this stage of their development (Blanco and de La Puente 2002). However, there was a somewhat surprising negative relationship between eye comb size and wing length. Yearling wings are unlikely to be fully developed during their first winter than mature males, however those with larger wings are likely to reach adult maturity first (Stewart 1963). In the minority of yearlings that invest in ornament displays early on, effort may have been diverted to displays, as opposed to body development, which may explain this negative relationship. Whilst these males may begin to build up dominance early on, they could experience negative carry-over costs later on (Lee et al. 2014, Kervinen et al. 2015). Alternatively, this investment strategy may be a terminal investment strategy, based on an internal prediction of shorter survival than average (e.g. Hanssen 2006). In any case, the exact causes of such a relationship are unclear and require further investigation.

To summarise, though patterns of relationship between health parameters and phenotype differ from adults, they do suggest covariance with condition for yearlings. The maintenance of at least a low level of condition-dependence may prevent males from investing highly into lekking, i.e. being heavy and with large eye combs. Overall, this may allow females to discriminate in their mate choice between lekking yearling males that have not undergone selection, and those top males that have survived and built lifetime lekking effort (Manning 1985, Schubert et al. 2007, Lifjeld et al. 2011, Kervinen et al. 2015).

3.5 Conclusions

The trade-offs between immunocompetence and ornament expression suggest eye comb size has a temporal interdependence with condition, a relationship which peaks during the breeding season. In addition, sexual signals grown on different time scales may function as multiple cues of condition, and also show heightened covariance during breeding. Overall, this suggests prolonged ornament expression is condition-dependent, heightending during the breeding season to further enforce honesty. In addition, as yearlings prioritise energetic investments into development, the condition-dependence of ornament expression limits their investment into lek displays. Overall this suggests only superior males can cope with investment into prolonged, faster and larger prebreeding ornament growth (McElligott et al. 2001), maximally expressing eye combs simultaneously with other exaggerated traits during the breeding season. Trade-offs between immunity and sexual advertisement are seen in many species during breeding (e.g. Kilpimaa, Alatalo and Siitari 2004). If trade-offs prior to this further constrain the ability an individual has to express an ornament, then investigating relationships between prebreeding ornament growth, condition and phenotypic quality may be widely important for understanding ultimate ornament elaboration and condition-dependence (Dmitriew 2011).

Chapter 4: The effects of condition-dependence on sexual ornament colouration across the spectrum

4.1 Introduction

Brightly coloured male ornaments are one of the most commonly observed, frequently-studied sexual signals in the animal kingdom, and also one of the most variable. Colours are produced in two main ways: structural colouration and pigment-based colouration. Structural colouration, such as melanin, produces colour additively via a specific integumentary microstructure that alters reflection (Prum and Torres 2003, Doucet et al. 2006, Andersson and Prager 2006). Pigments, such as carotenoids, are subtractive colourants deposited within structures such as feathers, wattles or combs, which modify colour by absorbing light, reducing reflectance in all or part of the colour spectrum (Bunkhardt 1989, Andersson and Prager 2006). However, it is normally a combination of both mechanisms that produces final colouration (e.g. Shawkey and Hill 2005, Mougeot et al. 2007a, Evans and Sheldon 2015). Carotenoid pigmentation has been a particular investigative focus in colour-based signalling, due to the complex condition-dependent relationships that brightly coloured ornaments have. Animals are unable to synthesise carotenoids but must sequester them from the diet; hence signal expression may rely on foraging ability (Olson and Evans 1998, Hill, Inouye and Montgomerie 2002 but see Evans and Sheldon 2013). Furthermore, carotenoids may have an immunological or antioxidant role, so only high quality males can cope with diverting them to ornament colouration, to have brighter or larger signals (e.g. Martínez-Padilla et al. 2007, Baeta et al. 2008, Alonso-Alvarez et al. 2008, Freeman-Gallant et al. 2011, Simons, Cohen and Verhulst 2012).

Carotenoid-based colouration has previously been studied in terms of human perception (e.g. Hill, Inouye and Montgmerie 2002, Kelber, Vorobyev and Osorio 2003, Montgomerie 2006). However, this approach is limited as many animals are sensitive to

reflected light outside of the human gamut, including ultraviolet (UV) spectra (Cuthill et al. 1999). Improved methods of measuring colouration have since been developed that are more objective, for example use of PCA analysis to summarize variation (e.g. Langkilde and Boronow 2010), or take into account bird-perceived colouration, e.g. summary metrics such as UV chroma, which allow more accurate study of signal perception (e.g. Bleiwess 2004, Mougeot et al. 2007a, Delhey and Peters 2008, Evans and Sheldon 2015). However, carotenoid-based colouration is, in reality, a composite trait, and not based on pigmentation alone (Shawkey and Hill 2005). It is composed of background reflectance; made up of a UV-reflective (usually white) background, determined by feather microstructure, and carotenoid deposits overlaying this, absorbing violet-blue light (400-500 nm) and causing traits to appear more yellow-red (Shawkey and Hill 2005, Mougeot et al. 2007b). Both components have their own sources of variance that may be linked to components of phenotypic quality (Mason 1923, Shawkey et al. 2006, Jacot et al. 2010, Evans and Sheldon 2015). Therefore, focussing on variation in colouration at wavelength peaks overlooks variance across the whole colour spectrum, and the full range of causes and mechanisms responsible for final colouration. As a result, the range and impact of variables, particularly condition, and their effect on colouration is not fully understood, which is important in understanding the information being communicated to conspecifics (Isaksson et al. 2008, Jacot et al. 2010).

An alternative approach is to investigate the bimechanistic function of carotenoid colouration using the full reflectance spectra, as opposed to narrowing it down to analysis of summary measures (Evans and Sheldon 2015). Several studies have investigated continuous colouration and found that by using the complete dataset for reflectance spectra they could analyse for wavelength-specific variation, such as that attributable to age or sex (Brunton and Majerus 1995, Hunt et al. 1998, Griggio et al. 2009, Evans, Summer and Sheldon 2012, Evans and Sheldon 2013 2015). In this method, reflectance spectra are partitioned into narrow (e.g. 2 nm) wavebands and each band analysed as a distinct trait. Sources of variance are modelled for each wave band and visualised as a

continuum in order to study across spectrum peaks in variance. These peaks can then be attributed to either of the mechanisms composing carotenoid-based ornaments; carotenoid pigmentation affecting violet-blue reflectance or background reflectance, which has broad-spectrum effects, particularly in the UV spectra (Mougeot et al. 2007b, Evans and Sheldon 2015). This allows us to unravel the sources of variation effecting either background reflectance or carotenoid pigmentation. However, it does not give a good account of the proportions in which such variables are important across the spectra (Dale 2006, Mundy 2006). Evans and Sheldon (2015) took this further by using a quantitative genetics approach to visualise the proportion of variance in reflectance attributable to environmental, additive genetic and annual variables, giving a more complete view of factors affecting total colouration. However, phenotypic variability has a condition-dependent element, meaning it may be affected by the multitude of variables that affect condition, such as mass, immunity and parasite burden (e.g. Mougeot 2008, Aguilera and Amat 2007, McGraw et al. 2009, Mougeot et al. 2010). Such variables have been found to affect colouration in investigations using summary metrics, but have been little studied as part of the whole spectra models. There is a lack of understanding as to the effect of condition-based measures on the whole spectra, and thus final colouration. Furthermore, ornament colouration can correlate with ornament size (e.g. Hill 1992, Hill and Montgomerie 1994, Pryke et al. 2002, Mougeot et al. 2007b, Yang et al. 2013), however the impacts of ornament size on the whole colour spectrum are also unaccounted for. Overall, there is a need for more comprehensive, descriptive surveys of the condition-based variables contributing to variance in colouration (Dale 2006, Siitari et al. 2007, Evans and Sheldon 2015).

We investigated the impact of condition on ornament colouration by modelling the wavelength-specific variation and directional effects attributable to condition-based variables in the red eye combs of black grouse (*Lyrurus tetrix*). Black grouse are a lekking species under strong intersexual and intrasexual selection, in which matings are skewed to a few top males (Alatalo, Höglund and Sutherland 1992, Kokko and Lindström 1997).

Males use a suite of morphological and behavioural traits as signals in intensive competition and mating on black grouse leks (Hovi et al. 1994, Rintamäki et al. 1997, 2000, 2001, Siitari et al. 2007, Hämäläinen et al. 2012, Höglund, Johansson and Pelabon 1997, Lebigre et al. 2013). Their red eye combs are the most dynamic of their ornaments, as they show considerable between- and within-year variance in size (Rintamäki et al. 2000, Kervinen et al. 2015, chapters 2 and 3). Previous studies show high testosterone levels are required for large growth, leading to trade-offs with immunity, hence comb size is condition-dependent (Rintamäki et al. 2000, Mougeot et al. 2007b, Chapter 2). The dynamic, integumentary nature of this ornament means comb size is also likely to affect comb colour, by the structures responsible for colouration altering as the comb enlarges. Combs are composed of a white skin-based background and multiple red dermal spikes. The spikes are likely to be carotenoid-pigmented, and therefore comb colouration has a condition-dependent basis, likely functioning in honest sexual signalling in grouse (Mougeot, Redpath and Leckie 2005, Mougeot et al. 2007a, b, Siitari et al. 2007, Pike et al. 2009, but see e.g. Fitze et al. 2007). In addition, testosterone may function as a lipoprotein upregulating carotenoid transportation and affecting colouration, further increasing the costliness of carotenoid-based colouration (McGraw, Correa and Adkins-Regan 2006, Martínez-Padilla et al. 2011). Expression of eye combs is important throughout multi-annual, year-round interactions for black grouse in order to gain and maintain dominance and, ultimately, reproductive success (Kokko et al. 1998, Rintamäki et al. 1999, Siitari et al. 2007). Therefore, using a longitudinal dataset of individual eye comb size across multiple years we develop previous multi-parallel waveband methods of modelling to investigate the variance in eye comb colour explained by a range of condition-dependent measures, and the directional effect they have, across the full colour spectra during a key breeding period.

4.2 Methods

Black grouse were studied at 13 lek sites, being captured from the winter flocking season (January-March) through to the breeding season (7 – 10 days in April/May) 2003-2008 using standard protocols (Alatalo et al. 1996, Siitari et al. 2007, Lebigre et al. 2012). These sites are located around Petäjävesi, central Finland (lat. 62°15′N, long. 25°00′E), and are all comprised of natural habitat for black grouse; European taiga forest edges surrounding a clear, open bog site. However, not all of the locations are completely natural, as peat-harvesting and/or black grouse hunting takes place on some of the sites, which can cause changes to population structure (Pekkola, unpublished data).

Birds were captured using oat-baited walk-in traps. On capture, each individual was ringed with a coded metal ring, and a range of morphometric and condition-based measurements recorded. For each individual, blood samples were taken from the brachial vein (Lebigre et al. 2007, Lebigre, Alatalo and Siitari 2013). An estimation of age for each bird as adult (≥2 years old) or yearling (1 year old) was recorded, based on differences in plumage colour and morphology (Helminen 1963). Young black grouse prioritise investment into physiological development as opposed to ornament expression, and ornaments are not fully-developed until the age of 2, hence only adults were included in our analyses (Kervinen et al. 2012, 2015),

Condition-based variables

Concentration of plasma immunoglobulins (IgGs) (standard unit (s.u.)) and weight (to the nearest 10g) were the two measures chosen for condition analysis, as they have both been previously shown to interrelate with eye comb size (chapters 1 and 2). IgGs are produced during humoral immune responses. In the presence of foreign substances, a host produces antibody-specific IgGs in order to cope with the immune threat (Lebigre, Alatalo and Siitari 2013). Replicates of blood to be analysed for IgG concentration were sampled at capture and frozen for later analysis. The methods, measurements and timings followed for blood sampling and IgG were based on those used by Lebigre,

Alatalo and Siitari (2013) and Pihlaja (2006), where full details can be found. Plasma was separated from red blood cells by centrifugation (12,000 rpm, 5 minutes) and frozen for later IgG analysis. Plasma samples were thawed and then immunoassayed using the ELISA method, to measure IgG concentration. Anti-chicken IgG antibody was used to coat ELISA plates, before incubating them overnight. Plates were emptied and then saturated with 1% bovine serum albumin prepared in a neutral phosphate-buffered saline solution, before carrying out plate washing with PBS-Tween. Dilutions of the same saline solution with the plasma samples for analysis were prepared as standards at 1:40,000 and 1:80,000 and incubated for 3 hours. Plate washing was repeated and samples incubated overnight with an alkaline phosphatase conjugated IgG. One final plate washing was carried out before adding an amine buffer (alkaline phosphatase substrate) to each well and incubating for 45 minutes. Optical density for each well at 405 nm was calculated against a curve generated by the standards for calibration, using spectrophotometry.

Eye comb size and reflectance

To calculate individual eye comb size, a digital video recording was taken of both eye combs against a scale. A still image of each eye comb was then taken from this image, and the area of the left and right eye combs (cm^2), respectively, measured using ImageJ software (Rasband 2012).Comb reflectance (Fig. 4.1) was calculated in the field using reflectance spectrophotometry (Ocean Optics USB2000) over the Galliform-visible range of 320-700 nm (Cuthill 2006). Reflectance was measured by placing the probe perpendicularly against the eye comb, such that the entrance to the fibre optic measurement probe was situated at a distance of 12mm (see Siitari et al. 2007 for detailed methods). The strobe illumination area encompassed an area measuring approximately 1.5 mm in diameter. This was replicated 4 times per comb per side for each individual sampled from 2003-2004, and 2 times per comb per side from 2005-2008, varying the probe position and side (left or right) measured on each repetition. After every four measurements, a 99% Spectralon white standard (LabSphere) was used to

recalibrate the spectrophotometer. The repeatability was calculated for 2003, and found to be relatively high (see Siitari et al. 2007).

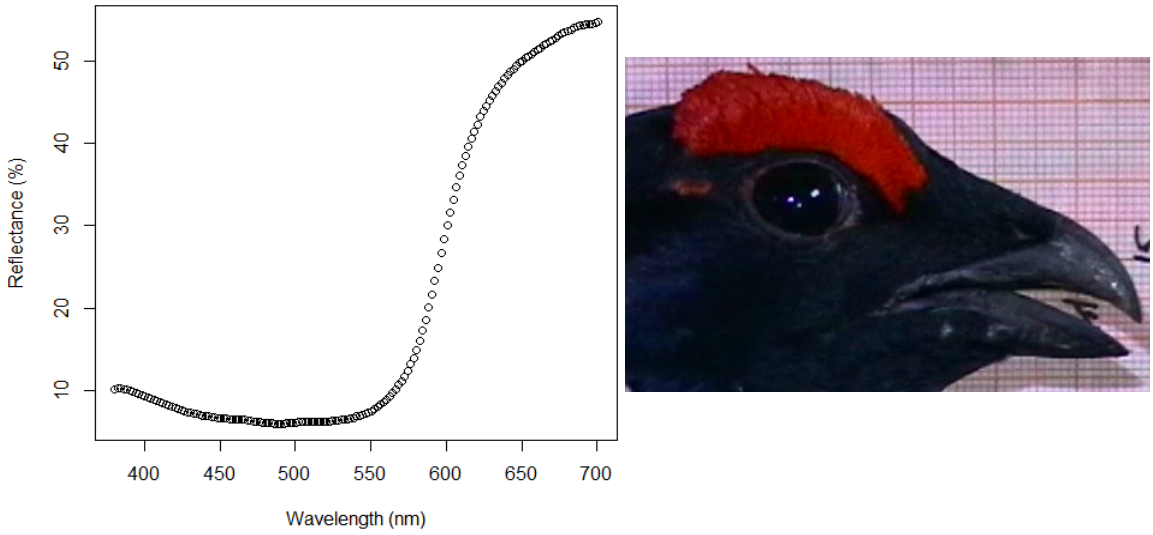

Figure 4.1: Example reflectance (%) for the eye comb of a black grouse male caught in late March.

Spectral compartmentalisation and statistical analysis

In order to investigate variation at specific wave bands, each reflectance spectrum was smoothed using a 30 nm moving average, before being partitioned into 2 nm wavebands by linear interpolation, and each analysed individually as a distinct trait (as per Evans and Sheldon 2015). This approach allows variation attributable to measured variables to be modelled at each narrow waveband and arranged sequentially to produce a visualisation of variance across the spectrum, allowing wavelength-specific effects to be depicted (Evans, Summer and Sheldon 2012, Evans and Sheldon 2015). This may be especially important for understanding carotenoid-based colouration as it allows, mechanistically, the influence of its two components, and the condition-based variance responsible, to be unravelled (Shawkey and Hill 2005, Evans and Sheldon 2015). This allows variation at each of the 190 x 2 nm wavebands to be modelled with condition-based variables, in

order to partition the phenotypic variance attributable to each across the spectrum. Our analysis only included readings in the range of 380-700 nm, as readings below this showed an unexplained shift, possibly due to measurement errors.

A global linear mixed model (LMMs) was used at each waveband to model phenotypic variance at that point in the spectra:

$$V_P = V_{IND} + V_{EYE} + V_{IGG} + V_{MASS} + V_{YEAR} + V_{SITE} + V_R$$

where variance equals that attributable to:

V_{IND}	=	individual differences
V_{EYE}	=	eye comb size
V_{IGG}	=	levels of IgG
V_{MASS}	=	body mass
V_{YEAR}	=	annual variance (random effect)
V_{SITE}	=	site (random effect)
$V_{RESIDUAL}$	=	error deviance

The variance was then placed in sequence in order to graphically represent the continuous variation in colour attributable to the modelled variables across the bird-visible spectra (Fig. 4.2).

In terms of choosing the fixed effect parameters; $V_{EYECOMB\ SIZE}$ was included to account for within-year temporal changes, and investigate relationships between size and colour; V_{IGG} levels and V_{MASS} have a condition-dependent relationship with eye comb size and therefore may account for variation in eye comb colour (chapters 2 and 3). In terms of random effects; V_{YEAR} was included to account for annual variance, such as climate; V_{SITE} to control for shared environment and $V_{RESIDUAL}$ to represent error deviance (e.g. Sheldon and Evans 2015, Mougeot et al. 2007b). Additive genetic variance was also initially included, via an animal model, similar to that used by Evans and Sheldon (2015). However, our pedigree dataset was significantly smaller and the loss of information in reducing our sample size was impacting our interpretation. The amount of variance explained by all parameters was broadly similar when additive genetic variance was

excluded; hence we excluded the pedigree information to allow a much greater sample size. Finally, whilst Evans and Sheldon (2015) included a temporal factor, Julian date was not included in the model as eye comb size and Julian date are strongly interrelated, hence only the most biologically relevant of the two was chosen. The final sample size used by the model was 280, with 1468 observations, which was repeated for each of the 190 x 2 nm wavebands.

In order to partition the phenotypic variance into variance attributable to predictors, we calculated variance estimates according to Evans and Sheldon (2015), with fixed effect variance calculated as marginal R^2 (Nakagawa and Schielzeth 2013). For each fixed effect we also calculate effect sizes as *r* (Cohen 1988), based on methods found in Nakagawa and Cuthill (2007), as our data was non-experimental and continuous. Finally, likelihood ratio tests were used to assess the significance of variance estimates, whereby the log-likelihood value of the full model is compared to that of a model in which the focal component has been omitted. The test statistic this produced, defined as twice the difference in log-likelihood between the two models, is assumed to follow a mixture of two chi-squared distributions, with zero and one degree of freedom, respectively (Self and Liang 1987, Evans and Sheldon 2015). All analyses were completed in R v.2.14.0 (R Development Core Team date 2012) using the lme4 package (version 1.1-1.7 and Bates, Maechler and Bolker 2015), with results based on restricted maximum likelihood.

4.3 Results

The results produced a graph of proportional phenotypic variance attributable to the fixed and random effects tested, allowing continuous variation and wavelength-specific peaks to be visualised. However, only by producing effect size graphs were we able to see additional importance of these variables, and the direction with which they affected reflectance.

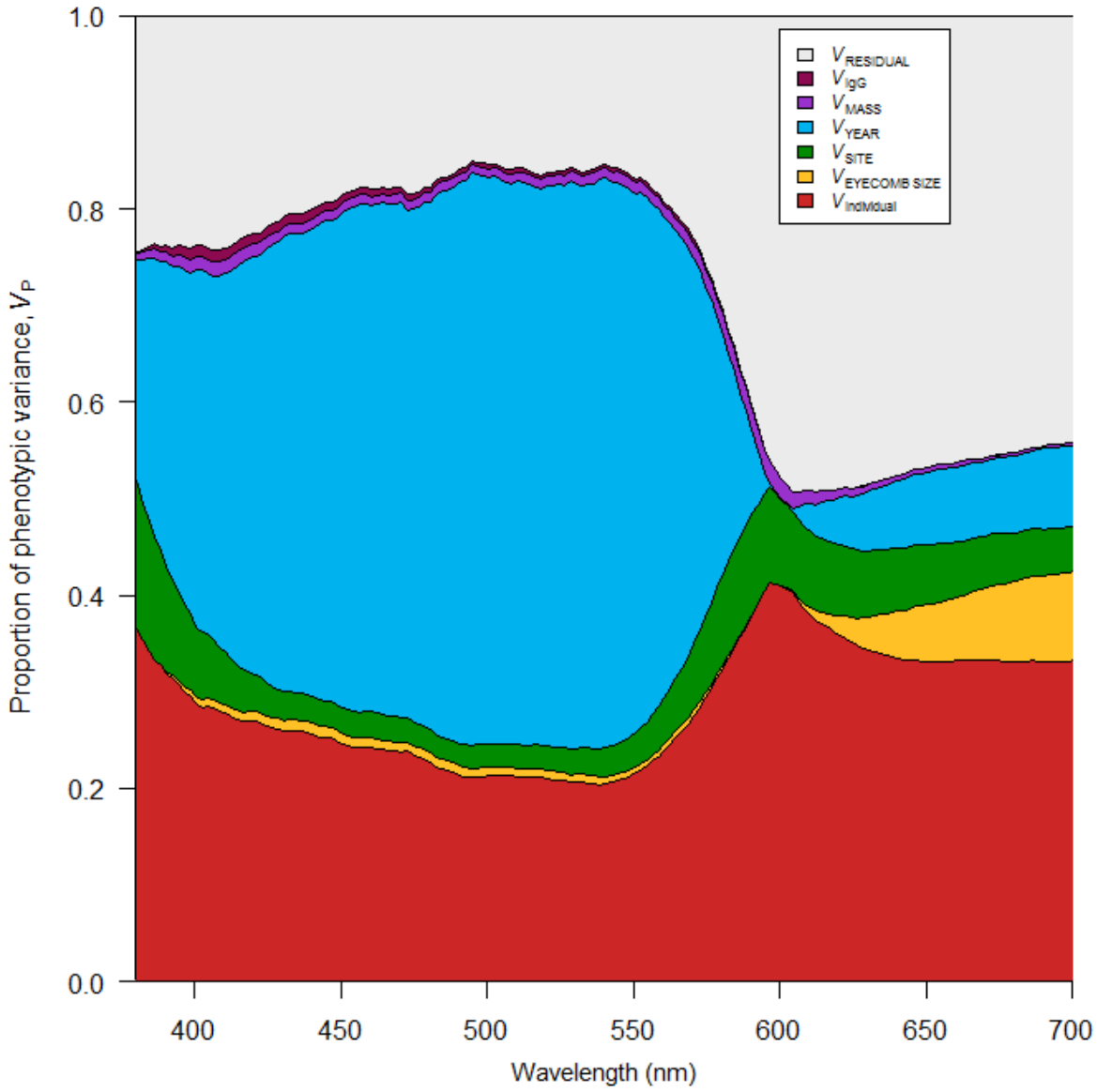

Figure 4.2: Proportional variance attributable to each of the fixed and random effects modelled for each 2 nm waveband from 380 – 700 nm of eye comb reflectance, and the remaining variance attributable to between-individual random effects and residual variance.

Fixed Effects

Changes in eye comb size explained the greatest proportion of variance in the yellow-red part of the spectra (Fig. 4.2). However, a significant effect of eye comb size was found across the whole spectra (Fig 4.3a); individuals with the largest eye combs had significantly increased reflectance in the violet-green part of the spectrum, but decreased red reflectance (Fig 4.3a).

In comparison, the variance in body mass and IgGs explained proportionally smaller amounts of variance. Body mass had a small but significant effect on colouration across the majority of the spectrum (up to 650 nm) (Fig. 4.2), but more strongly in the violet-green part of the spectrum (Fig 4.3b). Heavier males have increased reflectance in the UV-green part of spectrum, but reduced red reflectance (Fig. 4.3b). IgG concentration was only important in the UV-green part of the spectra, with no significant effect in yellow-red spectra (Fig. 4.2, 4.3c). Similar to mass, those with higher IgG concentrations showed increased UV-green reflectance.

Random and residual variance

In terms of random effects, year explained little variation in the orange-red part of the spectrum, but was responsible for the largest proportion of variance across the rest of the spectrum, particularly the blue-green spectra (Fig. 4.2, Fig. 4.4a). Site and individual variance also had a significant contribution to variance across the spectrum, peaking in the UV and the yellow-red spectra (Fig. 4.2, Fig. 4.4b and c). Finally, there was residual variance across the colour spectrum, with proportionally more unexplained variance in the yellow-red part of the spectrum than in the UV, blue and green part of the spectrum (Fig. 4.2).

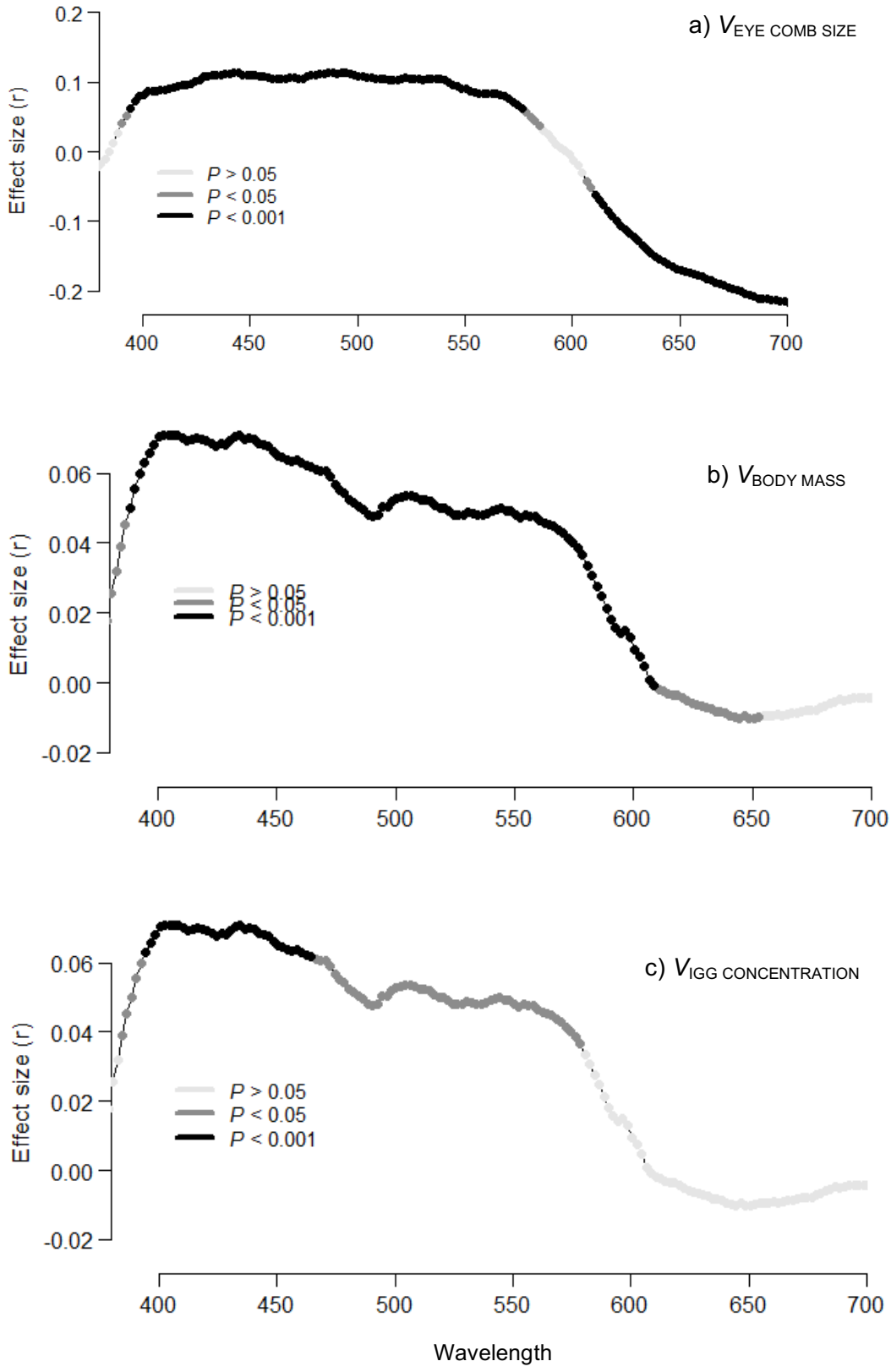

Figure 3.3: Effect sizes for fixed effects a) changes in eye comb size (cm^2) b) body mass (g) and c) IgG concentration (s.u.) modelled for each 2 nm waveband from 380 – 700 nm of eye comb reflectance

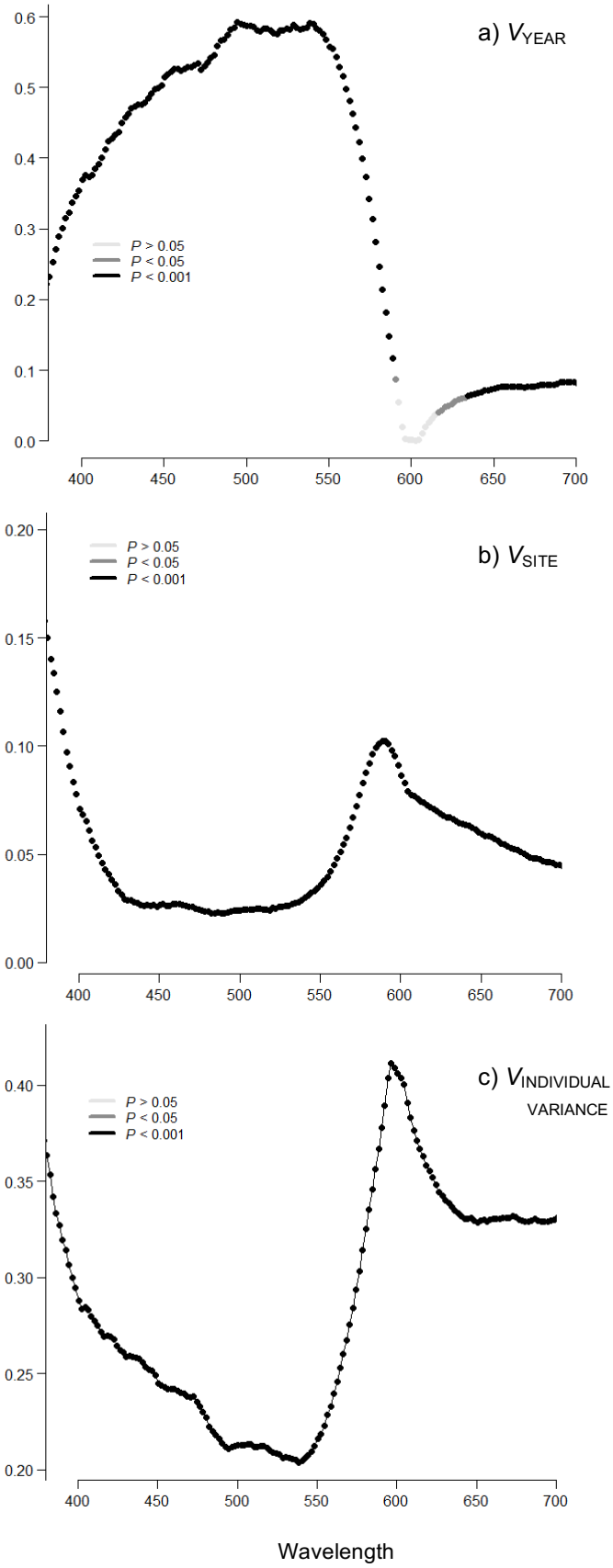

Figure 3.3: Effect sizes for random effects a) year b) site c) individual variance modelled for each 2 nm waveband from 380 – 700 nm of eye comb reflectance.

4.4 Discussion

Traditionally, animal colouration has been described either using measures based on human perception, i.e. tristimulus colour variables, such as hue, saturation and brightness (HSB) or based on only a fraction or summary of the animal-visible spectrum (e.g. Hill, Inouye and Montgomerie 2002, Kelber, Vorobyev and Osorio 2003, Delhey and Peters 2008, McGraw et al. 2009, Evans et al. 2010). More recent work uses the full, data-rich spectra to better understand the variables contributing phenotypic variance of colouration, from a mechanistic viewpoint (Evans and Sheldon 2015). The composite nature of carotenoid-colouration means that variation in wavelength may be attributed towards either the skin-based white background reflectance or foreground carotenoid-based redness (dermal spikes in black grouse), assisting in interpretation of wavelength-specific variance (Shawkey and Hill 2005, Mougeot et al. 2007b, Evans and Sheldon 2015). Furthermore, previous studies suggest bright signals are a condition-dependent signal of quality (e.g, Hill and Montgomerie 1994, Hill 2000, Senar, Figuerola and Domènech 2003). We developed this further using a novel method of spectral analysis to investigate in more detail the proportions of phenotypic variance that can be attributed to different aspects of condition and environment. By using effect sizes we were able to further analyse the importance and direction of largely condition-based variables in producing eye comb colouration.

Fixed effects

We found that the main driver of variance in comb colouration was the size of the eye combs. Eye combs can rapidly enlarge (chapters 2 and 3, Rintamäki et al. 2000), meaning the red dermal spikes, partly responsible for redness, reduce in density (e.g. in plumage; Keyser and Hill 1999). The dermal spikes become more widely ‘spread’ as the comb increases in size, and more of the underlying white skin may become visible (Soulsbury, unpublished data). As carotenoids are responsible for comb size, the carotenoid pigmentation will also become less dense, reducing absorption of reflectance in the blue-

green part of the spectra. However circulating carotenoids increase with decreasing body condition (e.g. reduced body mass and increased IgGs), for example during investment into ornament expression, which may counteract this negative effect (Lebigre, Alatalo and Siitari 2013). Black grouse eye combs differ to those of red grouse, which are more flat, hence this size-colour relationship within eye combs may function differently between Galliform species (Mougeot et al. 2007b). The overall effect is that red reflectance becomes reduced, and violet-green reflectance is increased, and the more visible background reflectance from the underlying skin may allow UV reflectance to increase (Mougeot et al. 2007b). Background reflectance is required to make red reflectance more visible, hence the composition of background reflectance and redness act to counterbalance one another.

Both testosterone and carotenoid-based colouration impose physiological trade-offs (testosterone; e.g. Mougeot et al. 2009, carotenoids; e.g. Aguilera and Amat 2007), and those with more attractive ornaments can usually better cope with this (e.g. heavier or with higher immunity; Blount et al. 2003). In chapters 2 and 3, our results suggested that eye comb size is traded off with body condition, specifically body mass and IgG concentration, via increased testosterone levels. Comb colouration, unlike size, is known to correlate negatively with testosterone (Siitari et al. 2007). Linked to this, we found both predictors of condition influenced UV-green reflectance positively and red reflectance negatively, similarly to eye comb size (although for IgG concentration this is non-significant in the red spectra), despite previous evidence suggesting background-reflectance may not be condition-dependent (Shawkey et al. 2006). Hence it appears condition-dependence of colouration, likely resulting from the trade-offs it is associated with, is manifested in UV-green reflectance, as opposed to the red. Interestingly, effects of both body mass (significantly) and IgG concentration (non-significantly) are positive in yellow spectra, suggesting these variables may have a further impact outside of eye comb size, in the composition of its colour.

Avian colour vision is tetrachromatic and so has 4 peaks of sensitivity (UV, blue, green and red), however this varies across species (Hart 2001, Cuthill 2006 and references therein). Grouse are sensitive to UV- and also, likely, red-reflectance which may be important in the perception, and thus evolution of, their sexual signal colouration (Rintamäki et al. 2002, Siitari, Viitala and Hovi 2002, Mougeot et al. 2005b, 2007a). Our results suggest condition is signalled in UV-green as opposed to red spectra, whereas some studies suggest near-UV reflectance relative to red reflectance has a negative relationship with condition (e.g. Mougeot et al. 2007b, Mougeot 2008), whilst others agree red/orange signalling may actually be negative (Rintamäki et al. 2002). This raises the question as to why black grouse eye combs are red, and ambient light availability may be the solution. Reflecting the predominant colours of ambient light makes an ornament more conspicuous (Endler 1992, 1993, Andersson, Örnborg and Andersson 1998, Théry 2006, Schultz, Anderson and Symes 2008). In early mornings, when grouse lek, ambient wavelengths are longer, hence red reflectance may become more important at this time (King, Kratochvil and Boyson 1997, Zagury and Fujii 2003). Grouse may even schedule their displays according to when they can be most conspicuous to conspecifics (Endler 1991, Olea et al. 2010); in lekking forest birds, e.g. Guinan cock-of-the-rock (*Rupicola rupicola*) who have similar bright orange ornaments, evidence suggests leks are chosen based on locations that allow optimal visual conspicuousness (Endler and Théry 1996, Heindl and Winkler 2003a and b). However, later in the day, when light is brighter, the shorter wavelengths become more predominant, and hence ornaments reflecting these wavelengths may become more important in sexual signalling (King, Kratochvil and Boyson 1997, Zagury and Fujii 2003). This supports the idea that both components of comb colouration, background reflectance and carotenoid pigmentation, have important functions in reflecting quality. The two components may even function as a within trait 'multiple-messages signal' (Candolin 2003, Evans and Sheldon 2015); indicating temporally or informationally different messages; for example the yellow hue of great tit (*Parus major*) colouration is associated with carotenoid content, whereas its yellow

chroma varies with body condition (Senar et al. 2008, also see Dreiss et al. 2008), or even average lifetime quality (Butler and McGraw 2011). The variation in ambient light may also link to the function of black grouse blue structural colouration (Siitari et al. 2007).

Overall, individuals may be able to optimize colourful signalling according to ambient light environment (Evans and Sheldon 2015). Both the red and the UV-green parts of the spectra may have important functions within this, possibly forming a 'multiple-messages' system, and the top males may be able to best cope with the trade-offs associated with ornament expression to display the most optimal combination of these components, as an honest cue of quality.

Random and residual variance

Our pedigree did not allow us to generate estimates of heritable variation in ornament colouration in the same quantitative genetic framework as the methods used by Evans and Sheldon (2015). However, we were able to model the variation down to differences between individuals, of which one variable would be additive genetic effects. Previous research suggests reflectance in the UV-blue spectra is heritable (Johnsen et al. 2003, Sheldon and Evans 2015). Conversely, contributions of individual variance in our results peaked in the UV and yellow-red part of the spectrum. Some evidence suggests reflectance in these parts of the spectrum is heritable (Kemp and Rutowski 2007); further study with a more extensive pedigree may reveal that UV and background reflectance is heritable for black grouse.

Of the random effects, a significant proportion of variance was attributable to both site and year. This was perhaps unsurprising as red colouration relies heavily on dietary carotenoids, and in poorer years or locations carotenoid availability may be reduced (Hill 1991, Grether, Hudon and Millie 1999, Hõrak et al. 2000, Isaksson, von Post and Andersson 2007), contributing to making the comb a good signal of current condition. Climate change may result in spring phenology differences (Ludwig et al. 2006); grouse rely on dietary carotenoids, which may largely come from spring bud burst, rather than

from fat stores, hence advancing spring phenology may have a significant effect on colouration during this period (Soulsbury, pers. comm., Heikinheimo and Lappalainen 1997, Møller 2008). Alternatively, for site variance, some locations may have a higher prevalence of parasites then others (Bensch and Åkensson 2003). Accounting for variance in parasites other than *Microfilaria* may thus explain some of the site or residual variance (Lebigre, Alatalo and Siitari 2013). Interestingly, there was a much higher proportion of residual variance in the redness region. Annual variance was also less important in this part of the spectrum. This may mean some other factor relates to the patterns of annual variation. For example, a better quality brood environment has been shown to positively affect condition and colouration later on (Evans and Sheldon 2012, Walker et al. 2013, Kervinen et al. 2015); mature males with these prior advantages may better cope with poor environmental conditions, e.g. due to having more energy reserves available (see Metcalfe and Monaghan 2001a for a review of brood environment effects), which may also account for a considerable proportion of residual variance.

An alternative source of residual variance may be measurer error. A longitudinal dataset such as this may have error in spectrophotometry measurements (Osorio and Ham 2002, Andersson and Prager 2006, Galván and Sanz 2010), possibly as a result of measurer error or variation. For example, the angle or distance the spectrophotometer probe is positioned from the comb may vary between measurers. Despite this, repeatability between measurements has been shown to be high (Siitari et al. 2007), and by using a large dataset with colour measurements taken across numerous locations, years and individuals, the effect of any errors may be minor.

4.5 Conclusions

We utilised a recently developed method to model the variance in a sexual ornament's bright colouration that could be attributed to condition-based parameters across the whole colour spectrum. By extending this method to analyse for effect sizes, we were able to expose further importance and directional effects of variables on reflectance. This

revealed the importance of ornament size in carotenoid-based reflectance. It also showed that both size and condition-dependence are manifested majorly in UV-green spectra, in a positive direction, as opposed to redness being the cue of quality, as is often suggested for carotenoid-based colouration. Despite this, we discuss both components being likely to have importance in sexual signalling, functioning interrelatedly to display highly conspicuous ornaments (e.g. Stuart-Fox and Moussalli 2007), as opposed to the redness being more important. Understanding the sources of variation affecting both components is likely to be in important in unravelling the information conveyed to conspecific (Shawkey et al. 2006).

Chapter 5: General Discussion

5.1. General discussion

The expression of dynamic, integumentary ornaments peaks during the breeding season, when interactions relating to dominance and reproductive success occur most intensely (Pérez-Rodríguez 2008). However, for many species, interactions and ornament expression outside of this are important in determining ultimate dominance and reproductive success (Bearhop et al. 2004, Tibbetts and Safran 2009). Despite this, little is known about the continuous dynamics of ornament expression, and the ability of differing males, e.g. in dominance or condition, to gain a competitive advantage by being able to cope with the costs of expressing ornaments over extended time periods.

Modelling of black grouse eye comb growth with condition- and dominance-related variables showed that scheduling, i.e growth rate, timing and elaboration, of ornament growth over the lead-up to during the breeding season was condition-dependent (Rands et al. 2011). In species such as black grouse that take part in intra- or inter-sexual interactions year-round, ornament displays outside of the breeding season may give a competitive advantage over conspecifics, and males vary in their ability to exploit this (Rintamäki et al. 1999, Peters, Astheimer and Cockburn 2001 Mougeot et al. 2005b, Reudink et al. 2009a, Laucht, Kempenaers and Dale 2010). Our results suggest expressing larger eye combs over a prolonged period is costly, likely as a result of the immunosuppressive nature of testosterone, which is maintained at high levels to increase ornament size (Rintamäki et al. 2000, Mougeot et al. 2009). Concordantly, black grouse adult males expressing larger combs lost condition over the winter-flocking and breeding season (chapter 3). Not only did top condition males have the resources to cope with this, maintaining large eye comb expression over an extend period (e.g. McElligott et al. 2001, Lindström et al. 2009), but these males also had the most elaborate static ornaments (lyre) during the breeding season. Displays of multiple traits are likely to enforce honesty, so, in species expressing a suite of dynamic and more static traits, top quality males may

cope with multiple trade-off costs to display quality in a number of ways, at optimal times or over extended periods (Candolin 2003). Heightened covariance of eye comb size with lyre length, and apparent loss of condition over the breeding season in males expressing large eye combs suggests condition-dependence and honesty may be further enforced during the most intense periods of sexual interactions.

Conversely, dominant adults had larger eye combs at all times, but this did not affect growth-scheduling. For species in which dominance status is achieved through year-round, multi-annual interactions, ornament expression may be important at all times (Kokko et al. 1998, Rintamäki et al. 1999, Reudink et al. 2009a, Laucht, Kempenaers and Dale 2010).

The relationship of condition-dependence and dominance with eye comb size during pre-breeding and breeding ornament expression was investigated separately in yearling males, due to age-related differences in reproductive investment. Young males of many species have different priorities for energetic investment to adults, focussing on physiological development as oppose to sexual displays (Kervinen et al. 2012, 2015). In line with this, only a minority of black grouse yearlings participate in dominance-related behaviours, hence dominance does not appear to be important in eye comb growth. However, condition-dependence of ornament expression in yearlings did seem to remain. Reproductive success in many species, such as black grouse, is a result of multi-annual interactions, so there may be a benefit to early expression. For young males who do invest in lek displays, the evidence we found of comb expression covarying with condition and lyre length suggests honesty of expression is maintained, although early, prolonged or accelerated growth may be costly.

Many elaborate ornaments that are dynamic in size are also brightly coloured. By using and extending a recently developed novel method (Evans and Sheldon 2015), we were able to investigate the condition-dependent relationships we had found in ornament growth further, in terms of colouration. The condition-dependence of adult eye comb size

was maintained in colouration, likely in a similar testosterone-based mechanism to that maintaining immune trade-offs of eye comb size. In addition the two signalling components of eye comb expression, colour and size, were highly interrelated. Human-based perception often expects redness or yellowness to be the important aspect of colourful carotenoid-based signalling, however, the direction of the size and condition-dependent relationships with eye comb expression were converse to this. Better condition, larger-ornamented males showed reduced comb redness, and increased UV reflectance. Considering the bimechanistic nature of the carotenoid-based ornament structure of eye combs, red dermal spikes overlaying white skin, it is likely the density of these spikes decrease with size, simultaenously increasing background reflectance, explaining the mechanism behind the reflectance pattern our results showed. However, it does not explain why eye combs are red but higher quality males reflected less redness. Previous studies have shown evidence for redness being both negatively and, respectively, positively related to colouration (Rintamäki et al. 2002, Mougeot 2008). The nature of the UV-inclusive bird-visible colour spectrum may mean ornaments are perceived differently by birds, and the importance of comb colouration may not be about red reflectance at all; it may, for example be about conspicuousness (e.g. Arai and Sato 2007, Stuart-Fox and Moussalli 2008), with the contrast between the redness and background reflectance being the key. We discuss this in the context of ambient light environment, as reflecting similar colours to the dominant ambient wavelengths is expected to increase conspicuousness, which some species exploit (e.g. Endler 1992, 1993). This may be true in black grouse; the red wavelengths that are dominant at the times when grouse lek may function to increase comb conspicuousness. Finding evidence of variance across the spectrum being important in a coloured signal affirms the importance of this novel method, revealing trends that may be missed or misunderstood when focussing on single peaks.

5.2. Conclusions

In conclusion, displays of large eye combs vary interdependently with condition, likely through the immunosuppressive costs of testosterone. Only top quality males are likely to have the resources available to afford the costs associated with expressing large ornaments on a prolonged basis. As dominance is a product of multi-annual, year-round interactions in many species, earlier or more elaborate prebreeding expression may provide a highly effective competitive advantage in improving dominance status and reproductive success. In addition, our results suggest that the same condition-dependent drivers of eye comb size may, alongside comb size itself, be the main drivers of carotenoid-based colouration. However, there is clearly much that is still unknown about carotenoid-based ornamentation. Many species engage in off-peak dominance-related interactions therefore, in order to understand the variance that affects sexual ornament colour and size and the information they convey, the full, data-rich temporal range and colour spectrum of ornament expression should be used. Carrying out investigations into individual differences in ornament expression in this way may be key to understanding the full dynamics of sexual signalling, and its impact on fitness.

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Appendix

Table A1: Significant and non-significant (<0.01) LMM model outputs for the relationship between Julian date, and condition and dominance. *Standard Error. **Linear ***Quadratic. ****Distance from the lek centre.

Model	Variable	Estimate	±SE*	*T*	*p*
	Adults				
Body mass (interaction) (345 individuals/ 233 data points	Intercept	21.86	27.84	0.79	0.433
	Julian date (l.**)	-3.34	1.07	-3.13	**0.002**
	Julian date (q.***)	0.02	0.00	2.74	**0.007**
	Body mass	-2.49	3.90	-0.64	0.524
	Julian date (l.) x body mass	0.46	0.15	3.09	**0.002**
	Julian date (q.) x body mass	-0.00	0.00	-2.64	**0.009**
Body mass (fixed effect) (233 individuals/ 345 data points)	Intercept	-60.54	10.59	-5.71	**<0.001**
	Julian date (l.)	-0.03	0.00	-5.32	**<0.001**
	Julian date (q.)	0.01	0.00	13.80	**<0.001**
	Body mass	9.05	1.48	6.10	**<0.001**
Lek attendance (interaction) (146 individuals/ 240 data points)	Intercept	3.94	0.40	9.10	**<0.001**
	Julian date (l.)	-0.03	0.02	-2.18	**0.030**
	Julian date (q.)	0.00	0.00	5.98	**<0.001**
	Lek attendance	0.47	0.47	0.98	0.326
	Julian date (l.) x Lek attendance	-0.01	0.02	-0.37	0.716
	Julian date (q.) x Lek attendance	0.00	0.00	0.62	0.534
Binary Lek attendance (fixed effect) (146 individuals/ 240 data points)	Intercept	3.97	0.29	13.73	**<0.001**
	Julian date (l.)	-0.04	0.00	-4.39	**<0.001**
	Julian date (q.)	0.00	0.00	11.23	**<0.001**
	Lek attendance	0.43	0.15	2.78	**0.006**
Binary copulations (interaction) (148 individuals/ 244 data points)	Intercept	4.30	0.33	13.03	**<0.001**
	Julian date (l.)	-0.04	0.01	-3.45	**<0.001**
	Julian date (q.)	0.00	0.00	8.43	**<0.001**
	Copulations	0.28	0.46	-0.62	0.538
	Julian date (l.) x Copulations	0.01	0.02	0.83	0.409
	Julian date (q.) x Copulations	-0.00	0.00	-0.83	0.409
Binary copulations	Intercept	4.15	0.28	14.95	<0.001

(fixed effect) (148 individuals/244 data points)	Julian date (l.)	-0.03	0.01	-3.79	**<0.001**
	Julian date (q.)	0.00	0.00	10.33	**<0.001**
	Copulations	0.05	0.16	0.34	0.734
Distance from the lek centre (interaction) (141 individuals/228 data points)	Intercept	4.72	0.42	11.22	**<0.001**
	Julian date (l.)	-0.05	0.02	-3.14	**<0.002**
	Julian date (q.)	0.00	0.00	6.98	**<0.001**
	Distance.****	-0.02	0.02	-1.39	0.165
	Julian date (l.) x Distance.	0.00	0.00	0.94	0.351
	Julian date (q.) x Distance.	-0.00	0.00	-1.00	0.318
Distance from the lek centre (141 individuals/228 data points)	Intercept	4.48	0.30	14.99	**<0.001**
	Julian date (l.)	-0.04	0.01	-4.19	**<0.001**
	Julian date (q.)	0.00	0.00	10.61	**<0.001**
	Distance.	-0.01	0.00	-1.88	0.062
	Yearlings				
Body mass (interaction) (345 individuals/ 233 data points	Intercept	-11.96	12.27	-0.97	0.33
	Julian date (l.**)	-0.12	0.51	-0.24	0.81
	Julian date (q.***)	0.00	0.00	0.44	0.66
	Body mass	2.03	1.74	1.16	0.25
	Julian date (l.) x body mass	0.01	0.07	0.17	0.86
	Julian date (q.) x body mass	0.00	0.00	-0.32	0.75
Body mass (fixed effect) (233 individuals/ 345 data points)	Intercept	-12.36	4.21	-2.94	**<0.001**
	Julian date (l.)	-0.04	0.00	-10.51	**<0.001**
	Julian date (q.)	0.00	0.00	20.55	**<0.001**
	Body mass	2.08	0.60	3.49	**<0.001**
Lek attendance (interaction) (146 individuals/ 240 data points)	Intercept	2.22	0.24	9.11	**<0.001**
	Julian date (l.)	-0.03	0.01	-5.09	**<0.001**
	Julian date (q.)	0.00	0.00	11.80	**<0.001**
	Lek attendance	0.35	0.48	0.73	0.468
	Julian date (l.) x Lek attendance	-0.02	0.02	-1.23	0.222
	Julian date (q.) x Lek attendance	0.00	0.00	1.77	0.078
Lek attendance (fixed effect)	Intercept	2.31	0.23	9.91	**<0.001**
	Julian date (l.)	-0.04	0.01	-6.45	**<0.001**

(146 individuals/	Julian date (q.)	0.00	0.00	14.83	**<0.001**
240 data points)	Lek attendance	0.02	0.16	0.16	0.874
Binary copulations	Intercept	2.24	0.22	10.08	**<0.001**
(interaction)	Julian date (l.)	-0.04	0.01	-5.90	**<0.001**
(148 individuals/	Julian date (q.)	0.00	0.00	13.11	**<0.001**
244 data points)	Copulations	-0.08	0.42	-0.20	0.841
	Julian date (l.) x Copulations	0.00	0.02	0.14	0.887
	Julian date (q.) x Copulations	0.00	0.00	-0.25	0.804
Binary copulations	Intercept	2.23	0.21	10.47	**<0.001**
(fixed effect)	Julian date (l.)	-0.04	0.01	-6.52	**<0.001**
(148 individuals/244	Julian date (q.)	0.00	0.00	15.51	**<0.001**
data points)	Copulations	-0.07	0.15	-0.49	0.626
Distance from the lek	Intercept	2.75	0.45	6.16	**<0.001**
centre (interaction)	Julian date (l.)	-0.06	0.02	-4.25	**<0.001**
(141 individuals/228	Julian date (q.)	0.00	0.00	8.74	**<0.001**
data points)	Distance.****	-0.01	0.01	-0.77	0.442
	Julian date (l.) x Distance.	0.00	0.00	1.30	0.196
	Julian date (q.) x Distance.	0.00	0.00	-1.77	0.079
Distance from the lek	Intercept	2.47	0.28	8.75	**<0.001**
centre	Julian date (l.)	-0.05	0.01	-6.56	**<0.001**
(141 individuals/228	Julian date (q.)	0.00	0.00	14.06	**<0.001**
data points)	Distance.	0.00	0.00	0.23	0.818

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